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EPIDEMIOLOGY AND CONTROL OF BANANA LEAF SPOT *(Mycosphaerella musicola* Leach*)* IN NORTH QUEENSLAND

By W. PONT, B.Sc.*

SUMMARY

The presence of Mycosphaerella musicola, **the ascigerous stage of** Cercospora musae **Zimm., in North Queensland is confirmed. Tip spotting is prominent on mother plants, while line spotting usually occurs on followers.**

The relationship of seasonal conditions and other factors to infection and duration of the incubation period is discussed in the light of field observations and experiments. Moisture and temperature have the most decisive influence during the epiphyllic stage and the greatest intensity of infection occurs during the wet summer months. The incidence is depressed during the winter when low temperatures and reduced humidity reduce sporulation and inhibit spore germination. Damage is at a minimum during the dry spring and early summer when copious dews permit a limited amount of infection only.

After penetration has taken place the interaction of temperature, intensity of infection and perhaps growth rate of the plant have to be considered. The period of latency before the streak stage appears is noticeably lengthened for infections which originate during the cooler and drier weather.

Two factors which are responsible for lengthening the apparent period of latency are spore dormancy and infection of older leaves.

The duration of the time taken for the streak stage to pass to the mature spot is dependent on intensity of infection and no effect of environment is clearly indicated.

Age of leaf has no influence on rate of spot development, at least while leaves are not apparently senescent.

Leaf spot control experiments conducted in North Queensland during the years 1951–1958 are described and discussed. The efficacy of white oil emulsion and malachite green mixed with either copper oxychloride, Bordeaux mixture or zineb is proved.

A limited amount of work with oil/copper mixtures for leaf spot control has indicated that when treatment with water-based mixtures is practicable it is preferable.

The role of white oil emulsion in the control of Mycosphaerella musicola **is considered. The striking effect produced appears to be due to a suppressive action linked with the physiology of the host. The oil is not fungicidal or fungistatic in the conventional sense.**

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I. INTRODUCTION

Cercospora musae, the conidial stage of *Mycosphaerella musicola,* was first recorded on *Musa sapientum* in Java (Zimmerman 1902) but did not attract attention as a major parasite of the banana until more than a decade later when it caused serious damage to plantations in the Sigatoka district in Fiji. It was probably present in Queensland in 1923 but the first authentic records from South Queensland are dated early in 1926. In December of that year it was reported in North Queensland. In 1933 it appeared in the West Indies and since then has been recorded successively from Central America, South America and Africa, so it can safely be said to be a threat to production in all major banana-producing areas.

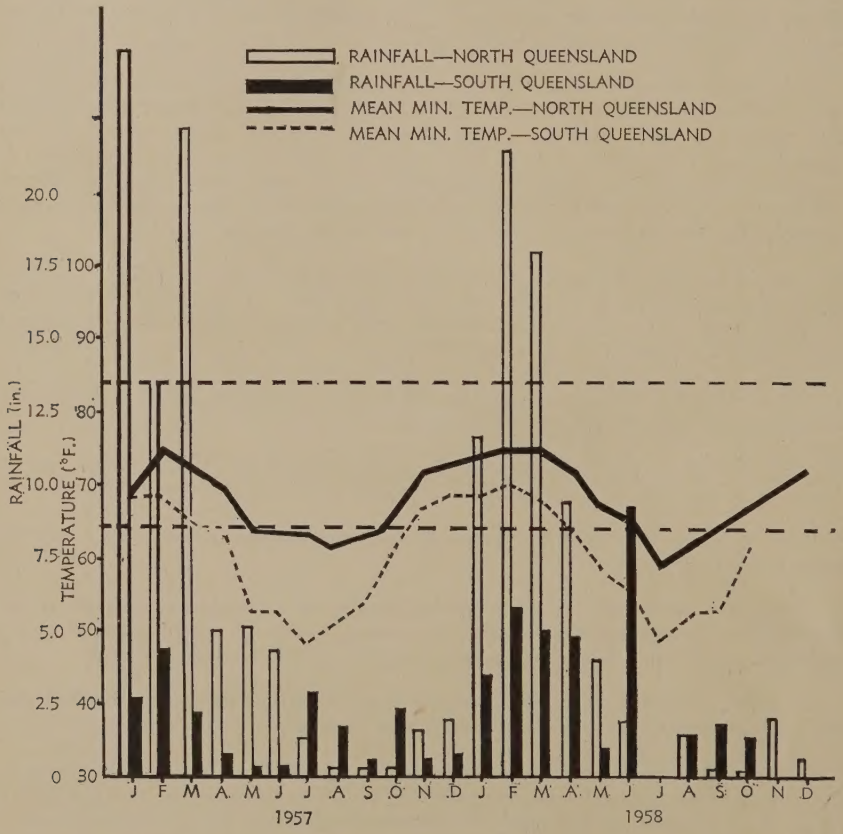

Fig. 1.—Climatic data for South Queensland (Brisbane) and North Queensland (Cairns), 1957-58. Horizontal broken lines mark off the temperature limits within which *M. musicola* is capable of 75 per cent. of its maximum growth.

Following the appearance of *Cercospora musae* in South Queensland, a considerable amount of work was done on the etiology of the disease and the effect of climatic factors on its development. The information gained was used to formulate a spraying schedule for that part of the State (Simmonds 1939). Because of the more favourable conditions, leaf spot proved to be a greater hazard in North Queensland banana plantations and in 1951 when investigations into its control were renewed it was decided to locate the experiments in the North. The major climatic factors contributing to differences in leaf spot incidence in South Queensland (Brisbane) and North Queensland (Cairns) are illustrated in Figure 1.

Concurrently with the control experiments, work was undertaken on the epidemiology of leaf spot under northern wet tropical conditions. The result of this work is discussed in the first part of the present article and a summary of the control experiments follows.

II. ECONOMIC IMPORTANCE AND HOST RANGE

Leaf spot infection first becomes apparent on affected foliage as minute chlorotic flecks which soon lengthen into yellow streaks 5 mm or more in length. These change colour to rusty brown, then broaden, become dark-brown in colour and develop a sunken centre which quickly dries out and becomes grey. The mature spots may be fusiform, elliptical or oval in shape. Their centres are studded with minute black sporodochia and they are encircled by dark-brown margins. On badly spotted leaves the majority of the spots do not reach a maximum size of 10 mm x 3 mm; however, isolated spots, particularly on water suckers, may be as large as 20 mm x 10 mm.

In North Queensland the streak stage may be first seen on the third, fourth or fifth fully expanded leaf, depending on plant growth and seasonal conditions. The rate of change from streak to spot is variable but mature spots are not often seen above the sixth leaf. Massed spotting in the mature stage quickly kills affected foliage, causing a progressive defoliation from the base of the plant upwards.

In this region of the State banana production is confined mainly to the coastal strip from Proserpine north to the Daintree River. Leaf spot is a major problem, however, only in the wet tropical zone which extends roughly from Ingham northwards. These coastal areas within the wet tropics are characterized by copious late-summer and autumn rains, giving a well-defined wet season early in the year. The wet season rains sometimes extend into the winter months but as a rule precipitation during the winter is much reduced and the spring and early summer are comparatively dry. Heavy dews are common during the drier part of the year.

Northern banana growers endeavour for economic reasons to manage their plantations so that the crop is harvested during the winter months. This means

that plants nearing the completion of their vegetative growth are subjected to the intense leaf spot infection which develops during and after the wet season rains. When the flower appears and leaf production ceases the plants are rapidly defoliated. Such a drastic reduction in the effective leaf area impairs the filling of the fingers, leading to reduced yields.

The bulk of the banana crop produced in the North is packed in cases as singles and consigned to southern markets by rail. A more insidious aspect of the leaf spot problem here is that consignments of fruit from plantations in which leaf spot damage is severe may arrive in the south in a "sprung" or mixed ripe condition and consequently be of little value. Exposure of the bunch following defoliation may also lead to premature ripening of the fruit while still on the plant. A bunch such as this is worthless and serves as a breeding ground for the banana fruit fly (*Strumeta musae* Tryon).

In new plantations, especially those with a reasonable degree of isolation, leaf spot may not become a problem until the plant crop and perhaps the first ratoon crop have been harvested. However, it is not uncommon to see severely damaged plant crops, particularly when these have been planted in proximity to sources of infection.

The varieties commonly grown commercially in North Queensland are the Cavendish and its mutants Mons Mari and Williams Hybrid. These are susceptible to leaf spot infection. The Lady Finger and Sugar varieties, which are grown on a comparatively minor scale, are also susceptible. The variety known in Queensland as Ducasses, which is of no commercial importance but is often grown in North Queensland and elsewhere as a windbreak, is immune. This plant is a natural hybrid which is believed to have arisen in Malaya, where it is still grown under the name of Awak Legor. It is a triploid made up of two genomes of *Musa acuminata* and one of *M. balbisiana* (F. W. Berrill, personal communication 1959).

The indigenous banana *Musa banksii* F. Muell., which is an inhabitant of rain-forests in the wet tropics, is susceptible but has only been observed to be infected when growing in the vicinity of infected plantations. The larger or yellow leaf spot (*Cordana musae* (Zimm.) von Hohn) is, however, commonly found on the wild banana and the native species is evidently an alternative host for this pathogen. At times first-year plantations established in rain-forest have been seen to be severely spotted by *Cordana musae* and it has been obvious that the infection originated from wild bananas nearby. It has been invariably noted that this parasite is quickly replaced by *Mycosphaerella musicola* in such plantations. In the first ratoon crop, or perhaps the second ratoon, *Cordana musae* plays a minor role only, being most often seen parasitizing the tissue around Cercospora lesions which have reached the self-limiting stage.

III. EPIDEMIOLOGY

(a) Spore Forms and their Importance

Mycospharella musicola Leach, the ascigerous stage of the banana leaf spot fungus, was first described from Jamaica (Leach 1941). Later the same author showed the difference in the manner of dissemination of the two spore

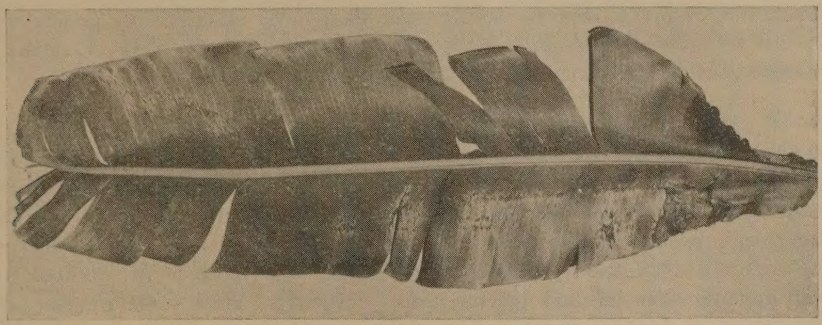

Fig. 2.—Line spotting on a leaf from a follower beneath a badly infected mother plant.

forms, conidium and ascospore, and its effect on the distribution of spots on affected leaves (Leach 1946). He demonstrated that conidial infection gave rise to line spotting (Figure 2) due to infection of the developing heart-leaf by the water-borne conidia, while infection of young fully expanded leaves by air-borne ascospores was responsible for tip spotting (Figure 3).

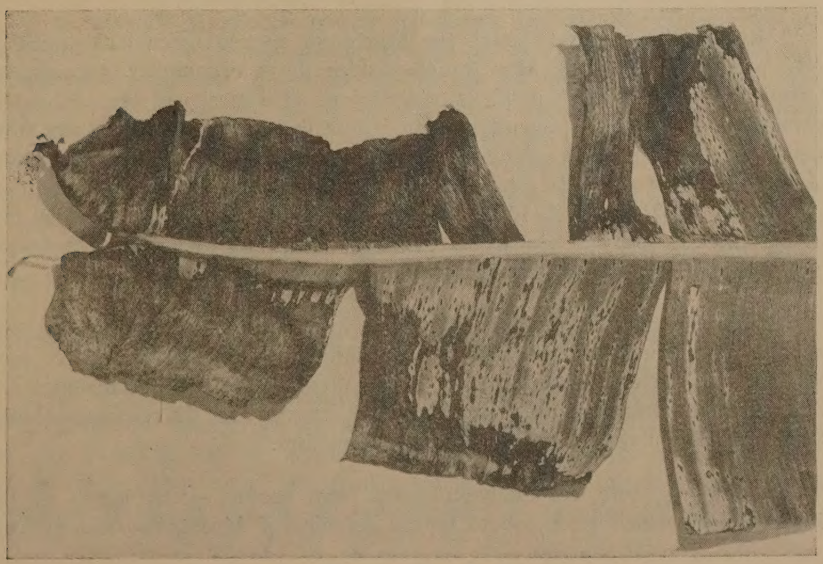

Fig. 3.—Tip spotting on a leaf from a mother plant.

Leach noted that with both types of spore secondary infection was rare. In Leach's work and in this paper also, the term secondary infection is intended to denote conidial infection of leaves other than the developing heart-leaf and ascospore infection of leaves older than the three youngest expanded leaves.

The isolation of the perfect stage of *Cercospora musae* has within recent years been confirmed in Cuba (Calpouzos 1955). Calpouzos found that the dimensions of the ascospores were greater than those described by Leach, viz. 18–20 x 4·5–5·6μ (Cuba) compared with 14–18·2 x 3–4μ (Jamaica). However, his measurements were made on germinated spores.

In North Queensland tip spotting as described by Leach is a common manifestation of leaf spot symptoms in banana plantations. In this region the ascospores of a related fungus (*Mycosphaerella musae* (Speg.) Syd.), the causal organism of a widespread and serious leaf speckle disease of bananas, are always present on the surface of banana leaves and it is easy to confuse the spores and infection processes of *M. musae* with those of *M. musicola* on leaf mounts from infected green leaves. However, after some practice the ascospores of the leaf spot fungus may be identified on such material because they are broader and their germ-tubes stouter than those of *M. musae*. The epiphyllic mycelium of the former is, in addition, less extensive, coarser and brown in colour. Penetration of the leaf in the case of *M. musicola* is quicker.

The isolation of spores of *M. musicola* for identification purposes is more difficult. The method employed to collect ascospores was to suspend spotted leaf pieces cut to fit the lids of 4 in. x 1 in. petri dishes over a water agar film in the bottom of the dishes or over agar-coated slides.

Most of the spores to reach the agar from spotted green leaf pieces of the Cavendish and Mons Mari varieties were those of another Ascomycete, *Leptosphaeria* sp. Limited numbers of ascospores of a species of *Mycosphaerella* were often seen in such preparations but it was impossible to isolate them from among the *Leptosphaeria* spores for purposes of identification. The association of *Leptosphaeria* with leaf spot lesions has been noted by previous authors (Simmonds 1933; Stahel 1937), and Calpouzos (1955) commented on the fact that the vast majority of ascospores obtained by the use of a similar technique from leaf-spotted material were multiseptate spores of this genus.

When spotted dead leaf pieces of the two varieties mentioned above were used, *Mycosphaerella*-type ascospores in addition to *Leptosphaeria* spores were obtained. However, perithecia of *M. musae* were present on this material and when the ascospores were plated out on potato dextrose agar the resulting colonies were invariably those of the leaf speckle organism.

Ascospores were eventually collected from spotted green leaves of the Sugar banana which gave rise to typical colonies of *Mycosphaerella musicola*

when transferred to potato dextrose agar. Measurement of these spores gave the following dimensions: range 14·4–19·2μ x 3·2–4·8μ; average of 50 spores 16·3 x 3.8μ. These agree well with those determined by Leach for *M. musicola* in Jamaica. It was found that in this work ascospores which had germinated on water agar were always considerably larger—up to 22·4μ x 6·4μ.

In Jamaica, Leach (1946) noted that ascospore infection is seasonal, occurring mainly in the autumn months. He observed that out-of-season tip spotting is common, however, on certain soils with "poor aeration, marked fluctuations in the oxidation/reduction conditions . . . and shallow tilth layers."

In North Queensland tip spotting (Figure 3) is prominent on mother plants, while line spotting (Figure 2) is usually found on followers as a result of heart-leaf infection by conidia washed from the overhanging leaves of the mother plants. Both types of infection are at a maximum during and following the wet season.

Commercial banana production here is mainly confined to flat or nearly flat land. Fertile alluvial soils or dark red loams derived from basalt are usually employed. There is no evidence that tip spotting is favoured by any one soil type but plantations on the deep, quick-drying basalt soils are generally considered to be more subject to leaf spot damage. This is probably because soil moisture drops quickly in these soils after the completion of the wet season rains and the reduced rate of leaf production due to this fact makes the disease more obvious.

(b) Seasonal Pattern of Disease Incidence

The seasonal variation in leaf spot incidence in North Queensland is shown in Figure 6 and is referred to again when discussing epidemiology and the results of control experiments. The data used were obtained from observations made throughout 1957 and 1958 on three unirrigated Cavendish stools in an isolated site at Kamerunga, in the Cairns district. As each new leaf emerged two rectangles were marked with a "Chinagraph" pencil on its lower surface. These were a 6 in. x 2 in. area in a central position on the left-hand side of the leaf (the side which unfurls first) and a 3 in. x 2 in. area in the region of the leaf tip, also on the left-hand side. The larger of the two rectangles was always situated so that its longest sides were at right angles to the midrib. All observations were then made within these marked areas. Readings were made each week when possible. Streaks and spots were marked as they appeared.

The monthly mean for intensity of infection plotted in Figure 6 was obtained by totalling the number of streaks which appeared within the marked areas on each plant during the month in question. The number of square inches within these datum areas was then calculated and the mean monthly figure, expressed as streaks per sq. in. was obtained by division. The numbers of

leaves involved each month in these calculations for each plant varied with the season and the growth rate of the plant and was greatest (maximum 7) during the first half of each year and least (minimum 3) during the second half.

It can be seen that there is a very noticeable lessening in leaf spot activity in the dry season. The seasonal pattern illustrated can be regarded as typical of the incidence of leaf spot throughout the banana-growing areas of North Queensland but the intensity of infection would be greater among large populations of plants, particularly on plantations in the wetter areas around Tully and Innisfail.

(c) Factors Influencing Infection

The effect of moisture and of the other factors listed below can be discussed in relation to germination and sporulation.

(i) Moisture

(1) *Effect on Germination.*—The effect of moisture on spore germination has been discussed by various authors. All agree that free moisture on the leaf surface is necessary for this process. In South Queensland, rainfall over a continuous 24 hr period is necessary to ensure abundant infection (Simmonds 1939). In the West Indies the important part played by dew as well as rainfall in the initiation of infection has been stressed (Stahel 1937; Leach 1946; Calpouzos 1955.)

In North Queensland, the wet season infection is undoubtedly initiated by rainfall. However, in the drier parts of the wet tropics, e.g. around Cairns, during the second half of each year periods of "effectual" rain are not common; the rainfall recorded is commonly in the form of storms or showers of much shorter duration, often during the daylight hours only. Such moisture quickly dries from the waxy banana foliage. On the other hand, heavy dews are usual during this dry period and it seemed probable that dew could provide the moisture needed to ensure infection at this time of the year.

In order to determine the effect of environment on infection during the dry part of the year a number of inoculations of Cavendish and Gros Michel plants was carried out during the second half of 1955. An aqueous suspension of conidia collected from leaf spots which had been incubated overnight in a humid atmosphere to induce sporulation was applied with a nebulizer to 3 in. x 2 in. rectangles marked on the lower surfaces of the leaves. Up to seven leaves on each plant were inoculated on occasions but usually five were used. Certain of the inoculated leaves were enclosed in plastic sleeves for 36 hr and the interiors of the covers and the leaves themselves were misted with water prior to enclosure. The results of some of these inoculations are summarized in Table 1. Rainfall and dates are given for the period during which wet weather could have influenced the particular inoculation.

TABLE 1

Results of Artificial Inoculation Experiments, Dry Season, 1955

Inoculated	Streaks Appeared	Incubation Period (days)	Wet Days and Rainfall (in.) to within 3 Weeks of Streak Appearance
July 18	Aug. 23	36	July 19 (0·02) ; 21 (0·05) ; 24 (0·02) ; 27 (0·03)
July 18*	Aug. 23	36	ditto
July 25	Aug. 29	35	27 (0·03)
Aug. 1	Sept. 13	43	Aug. 21 (0·04)
Aug. 1*	Aug. 29	28	Nil
Aug. 8	Sept. 13	36	Aug. 21 (0·04)
Aug. 8*	Sept. 13	36	ditto
Aug. 29	Oct. 7	39	Aug. 29 (0·14) ; 30 (0·20) ; 31 (0·86) ; Sept. 1 (0·18) ; 2 (0·24) ; 4 (0·05) ; 11 (0·14) ; 12 (0·52) ; 13 (0·13) ; 14 (0·04) ; 15 (0·14)
Sept. 13	Oct. 19	36	Sept. 13 (0·13) ; 14 (0·04) ; 15 (0·14) ; 18 (0·38) ; 19 (0·12) ; 22 (0·03)
Dec. 14	Jan. 16	33	Dec. 15 (0·03) ; 18 (0·80)

* Enclosed in plastic cover after inoculation.

It was obvious from the results of these experiments that infection could be independent of rainfall during these months. During the period from July 18 to August 8 four successful series of inoculations on uncovered leaves were accomplished. As the minimum observed period from germination to streak appearance is in the vicinity of four weeks, even during the wet season months, the only rain which could have affected the fate of these inoculations during the weeks from July 18 to September 13 occurred on five days with a maximum fall of only 0·05 in. Heavy dews were recorded during this dry period and in some cases dew was noted on inoculated foliage as late as 10 a.m. No consistent differences showed between covered and uncovered leaves.

In 1958 more information on the effect of dew on infection was obtained from two small experiments carried out at Kamerunga. In the first experiment two plants, one sheltered in a lath-house and the other in the open, were inoculated from the same stock spore suspension. The inoculum was applied within circles 4·5 cm in diameter marked on the lower surfaces of the three youngest fully expanded leaves. Three such circles were randomized on each leaf. The streaks were counted as they appeared. The following are the results expressed as mean number of streaks per circle:

Leaf position	1	2	3	Mean
Field plant	137	132	159	143
Sheltered plant	28	23	18	23

The plant in the open was exposed to only 0·04 in. of rainfall on 2 wet days from the date of inoculation (June 25) to July 29, when the first streaks were noted. Heavy dews were common during this dry period. Moisture visible to the naked eye was not at any time noticed on the lower surface of the inoculated foliage on the sheltered plant.

This evidence, however, does not exclude the possibility that some effect on the metabolism of the plant due to growth under conditions of partial shade could have contributed to the reduced infection on the sheltered plant.

In the second experiment the five youngest expanded leaves on a small plant in the open were inoculated with conidia on September 2, 1958. As before, the spores were applied within circles 4·5 cm in diameter, two of which were located on the lower surface of each leaf. The results of these inoculations first became evident as flecks in some of the inoculated circles on October 2. On October 9 an average of 80 streaks per circle was visible and by November 5, when the majority of streaks had become necrotic spots, an average of 86 lesions per circle had been counted.

From the date of inoculation until the time when the first streaks were counted the rainfall recorded was 0·20 in. on September 24, 0·15 in. on September 26 and 0·09 in. on October 2. None of these falls were early enough to have affected the issue.

A 7-day dew recorder (Theis and Calpouzos 1957) was used to record the occurrence of dews during the first fortnight after inoculation. This instrument recorded an average of 9 hr dew per night during this time. Evaporation of moisture from the glass plate of the instrument was complete on all mornings by 8 a.m. However, dew was seen on the protected lower surfaces of the inoculated leaves as late as 10 a.m.

These results suggest that dew can provide the free surface moisture which is needed to ensure infection by leaf spot spores. It must therefore be considered as a factor in the epidemiology of the disease, particularly in those banana growing areas in the North where long rainless periods are common during the dry season. In the wetter areas around Innisfail and Tully, rainfall in the second half of the year is greater and periods of "effectual" rain are more common. Nevertheless, in spring and early summer rainless periods of three weeks or more do occur during which copious dews are probably responsible for infection (Figure 1).

(2) *Effect on Sporulation.*—The part played by water—either rain or dew—in inducing the production of conidia has been well stressed (Stahel 1937; Simmonds 1939; Leach 1946). In addition, it has been demonstrated (Calpouzos 1955) that conidia are produced *in vitro* in atmospheres with relative humidities of 98 and 100 per cent. In the course of the work described in this paper conidia of *Mycosphaerella musicola* were produced, whenever required, by the exposure of leaf spots to a saturated atmosphere. It would be unusual if the incidence of

atmospheric humidities as high as those quoted above was not associated with either rainfall or condensation. Calpouzos (1955) has concluded therefore that, for all practical purposes, sporulation *in vivo* occurrs only when free moisture is present.

(ii) Temperature

(1) *Effect on Germination.*—The growth-temperature relationships of *Mycosphaerella musicola* have been investigated in detail (Simmonds 1933; Calpouzos 1955). Simmonds found that after incubation for 24 hr the optimum temperature for the germination of conidia was as high as 84°F. However, at lower temperatures germination increased with time and a fair percentage of spores could be expected to germinate even at temperatures as low as 59°F after exposure for 48 hr. Calpouzos determined 77°F to be the optimum for germination but also obtained excellent germination at 59°F after 48 hr.

According to Simmonds the optimum temperature for germ-tube growth was intermediate between that for growth of the fungus in culture (78°F) and that for germination (84°F).

A further study was made of the relationship of temperature to germination and germ-tube growth of conidia of *M. musicola* and the results are illustrated in Figure 4. Cercospora spores were collected from mature leaf spots which were exposed to a saturated atmosphere overnight; they were suspended in banana infusion broth and the suspension was used to prepare hanging-drop

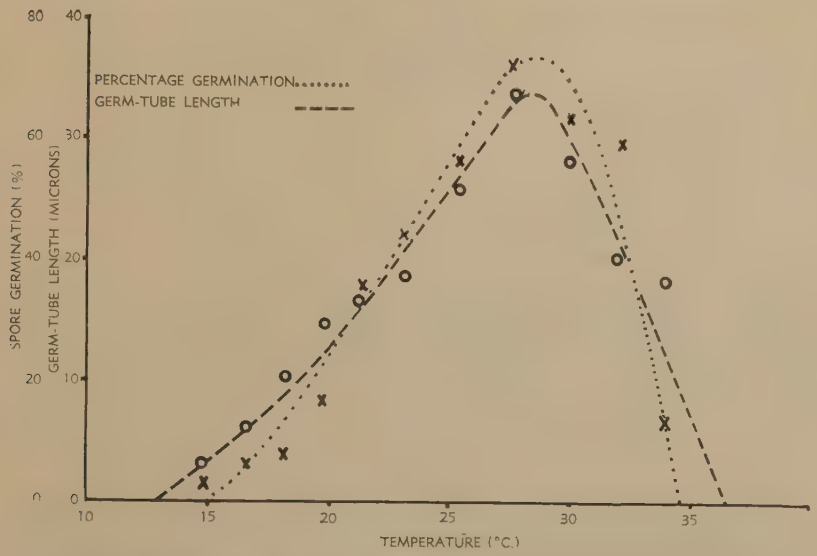

Fig. 4.—Relationship of germination and germ-tube growth (conidial) to temperature.

cultures in van Tieghem cells. The cells were then incubated in each of 15 compartments of a a multi-temperature incubator at temperatures ranging from 70°C to 34°C. After incubation for 24 hr they were removed and placed in a refrigerator overnight. Counts and measurements were made during the next day. Fifty spores were counted per hanging drop and measurements were made of the lengths of the longest germ-tubes on each of the germinated spores in these lots of 50. The figures plotted in the graph are means calculated from counts and measurements made by two observers.

It can been seen that the optimum temperature for both germination and germ-tube growth was 28°C (83°F) or a little higher. There was no germination below 15°C (59°F) in 24 hr.

In coastal areas of North Queensland the minimum temperature drops below 60°F usually only occasionally even in the coolest months of June, July, August and September (Table 2). Conditions are roughly similar to those in Jamaica, where "it is doubtful whether temperature ever falls low enough to prevent germination completely in any of the main banana growing areas . . ." (Leach 1946). However, in North Queensland the fact that the mean 10 p.m. to 6 a.m. temperatures do not exceed 70°F during these months indicates that low temperatures could be responsible for slowing up the processes of infection at this time of the year. The position is not comparable with that in South Queensland, where for long periods during the year nocturnal temperatures fall well below 60°F (Figure 1).

TABLE 2

Mean Monthly Night Temperatures (°F) for Cold Months, Kamerunga 1955–1957

(Thermohygrograph Readings)

Time of Reading	June	July	August	September
10 p.m.	67	66	67	69
2 a.m.	65	64	64	66
6 a.m.	64	63	62	68
Mean monthly minimum	63	62	62	64

(2) *Effect on Sporulation.*—Temperatures exert a marked effect on the production of conidia by *Mycosphaerella musicola.* In Jamaica the percentage of viable conidia formed on leaf spots each night is largely dependent on the prevailing night temperatures (Leach 1946). Leach considered that "the effect of low temperature on sporulation accounts for the consistent island-wide reduction in conidial infection which occurs every year in January and February."

In the work discussed here the variation in conidial sporulation with temperature was investigated in the following manner: Suitable spots were removed from leaves, placed in petri dishes in a saturated atmosphere and exposed to a range of temperatures in a multi-temperature incubator. Usually two dishes

each containing 4 or 5 spots were placed in each compartment. After 24 hr the spots were examined with a dissecting microscope and each was given a rating both for number of sporing sporodochia and for intensity of sporulation on the following scale: 0, nil; 1 trace to 25 per cent.; 2, 26–50 per cent.; 3, 51–75 per cent.; 4, 76–100 per cent. When the examinations commenced the dishes were removed from the compartments of the multi-temperature incubator and stood on an open bench at room temperature with the lids removed. The ratings were made by two observers on each occasion and the results were consistent.

In Figure 5 the results of two such experiments are used to illustrate the effect of temperature on sporulation. Means have been calculated for number of sporodochia and intensity of sporulation.

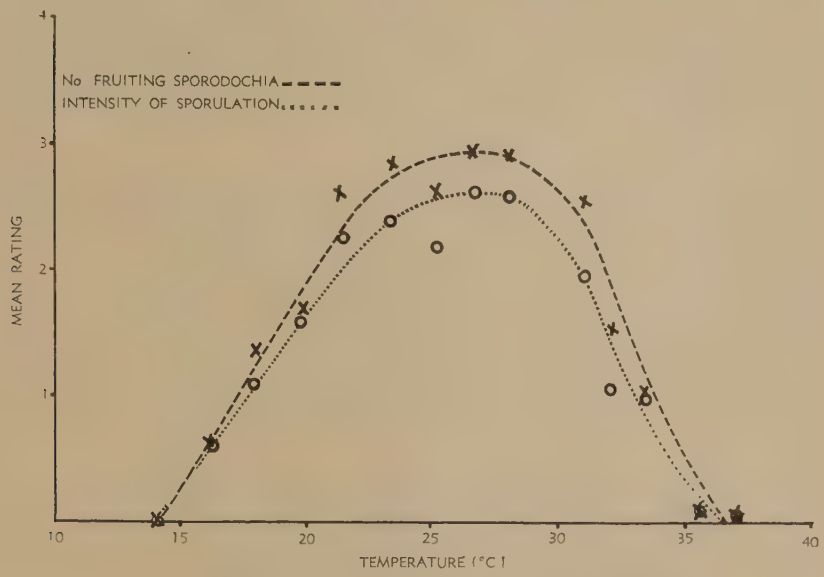

Fig. 5.—Relationship of conidial sporulation to temperature.

It can been seen that in a saturated atmosphere temperature affected both these variables. Both were negligible below 60°F and above 96°F. The temperatures most conducive to sporulation were in the range 70°F to 88°F and the optimum appeared to be around 83°F, i.e. very close to the optimum temperature for germination.

The mean monthly figures given in Table 2 indicate that the depressing effect of temperatures below 70°F on conidial sporulation could contribute materially to the reduced incidence of leaf spot in North Queensland during the second half of the year. It must be stressed, however, that in this part of the

State sporulation *in vivo* is not completely inhibited by exposure to night temperatures below 60°F. For example, during an unusual spell of cold weather in July 1959 which lasted for nine days the mean figures for the 10 p.m., 2 a.m. and 6 a.m. readings at Kamerunga were respectively 61, 57, and 52°F. On every morning during this period banana foliage was drenched with dew and it was possible to locate spots on which partial sporulation had occurred and from which mature conidia could be removed by stroking with a camel-hair brush.

(iii) Age of Leaf and Surface of Leaf

(1) *Effect on Infection.*—In Surinam, conidial infection of leaves older than the second fully expanded leaf was considered to be unimportant on the Congo variety (Stahel 1937). In Jamaica, the bulk of the conidial infection on the Gros Michel variety was shown to originate on the unfolding heart-leaf, while ascospore infection was unimportant on leaves older than the first three (Leach 1946). In South Queensland, however, quite extensive conidial infection of leaves other than heart-leaves was demonstrated on the Cavendish banana (Simmonds 1939).

During 1955 and again in 1959 a series of inoculation experiments on Gros Michel and Cavendish bananas was carried out to determine whether conidial infection of older leaves was possible under North Queensland conditions. The results of some of these inoculations are presented in Table 3. The conidia were collected from mature leaf spots and applied in aqueous suspension with a nebulizer to rectangles measuring 3 in. x 2 in. or to circles 4·5 cm in diameter on either the upper or the lower leaf surface. An attempt was made to ensure the application of approximately equal amounts of inoculum to each of the inoculated areas in any one experiment. The resulting intensity of infection is expressed in number of streaks per sq. in.

It can be seen that infection of older leaves on both Cavendish and Gros Michel varieties resulted from the application of conidia to the lower surfaces. On Plant 7, which was carrying a young bunch when it was inoculated, infection of the second and third leaves was obtained quite readily.

In Jamaica, the antibiotic effect exerted on leaf spot spores by miscellaneous epiphyllic mycelia has been considered responsible for the resistance of old leaves to infection (Leach 1946). In the tests described above no attempt was made to remove the mycelia except in the case of Plant 7 (upper surface).

There appears to be no reason why infection of older leaves by either conidia or ascospores should not be possible under plantation conditions in North Queensland. Some confirmation of this view has been provided by the fact that freshly germinated ascospores and new infections have been seen on leaf pieces taken from leaves in the sixth fully expanded leaf position showing both streak and spot stage lesions of the tip spotting type. Additional evidence of the existence

TABLE 3

Artificial Infection of Leaves of Gros Michel and Cavendish Banana
(Streaks/sq. in.)

Variety	Plant No.	Date of Inoculation	Surface Inoculated	Heart-leaf	Fully Expanded Leaves						
					1st	2nd	3rd	4th	5th	6th	7th
Gros Michel	1	18. vii. 1955	Lower ..	..	9	20	10	33	17	..	..
			Upper ..	..	0	1	1	4	1	..	..
„ „	2	29. viii. 1955	Lower ..	22	36	22	33	15	21	7	7
			Upper ..	..	1	0	1	0	0	2	0
Cavendish ..	3	18. vii. 1955	Lower ..	..	39	39	15	20	15	..	..
			Upper ..	..	7	1	1	1	0	..	..
„	4	12. ix. 1955	Lower ..	..	6	13	9	4	6	..	..
„	5	13. ix. 1955	Lower ..	14	15	20	7	10	4	..	..
„	6	14. xii. 1955	Lower ..	..	13	10	11	10	5	..	..
„	7	18. vii. 1955	Lower ..	..	..	24	21	..	..	..	..
			Upper ..	..	..	8*	0	..	..	..	..
„	8	6. ii. 1959	Lower ..	..	68	33	36	20	..	..	..
„	9	1. v. 1959	Lower ..	..	28	26	32	..	..	..	..
„	10	1. v. 1959	Lower ..	..	11	22	16	9	9	..	..
„	11	1. v. 1959	Lower ..	..	36	11	15	7	..	..	..

* Inoculated area rubbed well with damp cotton-wool pad prior to inoculation

of "secondary" infection was obtained in a leaf spot control experiment (Experiment 4, Stoney Creek) in which the incidence of the disease was significantly reduced by late cover sprays which were continued after the plants had flowered and leaf production had ceased.

The fact that leaf spot infection originates much more readily on the lower surface of a banana leaf than on the upper surface has been discussed by many authors. Stahel (1937) noted that the infection ratio between upper and lower leaf surfaces was much lower than one would expect from a comparison of numbers of stomata on these two surfaces. Leach (1946) suggested that there was a hydrotropic response by germ-tubes towards open stomata and concluded that the higher rate of infection of stomata on the lower surface could be attributed to the facts that stomata on this surface opened earlier in the morning and that the microclimate of the lower leaf surface was favourable to germ-tube growth for longer periods.

The results shown in Table 3 indicate that infection through the upper leaf surface is unimportant in North Queensland. The inoculations made on Plants 1, 2, 3 and 7 in 1955 resulted in an average intensity of infection of 21·2 streaks per sq. in. on the lower surface, compared with only 1·5 on the upper.

(2) *Effect on Sporulation.*—In North Queensland, conidia have been collected from the dead trash which hangs about the base of an affected plant. However, sporulation is most prolific on spots on living leaves. Although the production of conidia does not cease immediately a leaf dies, the capacity to produce these is apparently lost fairly soon after death, for they are usually not found in quantity on any except the two or three leaves last to die. An alternative possibility is that these spores are produced before the leaf dies but have not been disseminated.

Sporodochia develop on both upper and lower surfaces of spots. Those on the upper surface are the first to become evident and on narrow linear spots these appear to be more numerous. On broader spots the sporodochia on the lower surface are smaller and more numerous than those on the upper surface. Conidia are produced freely on both surfaces when environmental conditions are favourable.

The results reported here relating to the effect of certain variables on infection by the banana leaf spot fungus have been derived from experiments and observations involving only the asexual spores of the pathogen. Because of the difficulties, mentioned previously, involved in isolating the ascospores of *Mycosphaerella musicola* from the spores of other Ascomycetes commonly found on banana leaves in North Queensland, it has been impossible to obtain any precise information of a similar nature about these spores. However, it is assumed that the moisture and temperature requirements for sporulation, germination and infection are closely similar for the two spore types.

(d) The Developmental Periods

The manner in which the leaf spot fungus infects a leaf was first described by Stahel (1937). The essential processes involved in the establishment of infection may be briefly described as follows: Following on the germination of the spores and after a certain period of epiphyllic existence, said by Stahel to last 4–8 days, the germ-tubes form appressoria over stomata and penetration of the leaf takes place through the stomatal pores to the air chambers. The infection hyphae eventually spread to neighbouring air chambers and travel through the spongy parenchyma to the palisade tissue. About this time the streaks become visible.

As the streaks age, epiphyllic mycelia of the fungus emerge from stomata on both surfaces, extending for some distance around the streak. Infiltration of the tissue around the streak then ensues and the fungus re-enters the tissue through the stomatal pores. Necrosis then takes place, the sporodochia are initiated below stomata in the brown area and the typical sporing spot appears.

There are thus two obvious stages in the development of a typical leaf spot, viz. the streak and the sporing spot. Associated with these are two critical periods of development—firstly, the incubation period, which is the time that elapses between germination of a spore and the appearance of a streak; and secondly, the period during which the change from streak to sporing spot takes place.

The effect of environment on the duration of development was first investigated by Simmonds (1939) in South Queensland. He found: "The effect of temperature is fairly well defined. The length of the incubation period required before streaks develop increases with decrease in temperature." The incubation period in South Queensland was calculated to vary from a minimum of approximately a month in summer to a maximum of $3\frac{1}{2}$ months in winter. No attempt was made to effect a correlation of incubation period with plant growth rate, which in South Queensland is bound up with seasonal temperature. In his observations this worker found that streaks appeared in peaks of development subsequent to the first appearance. He noted that the length of the incubation period did not in many cases allow this phenomenon to be attributed to germination in a second period of more suitable weather and suggested as a probable explanation that "the fungus is present within the tissues in an inactive or latent condition, and requires a lowering of resistance on the part of the host such as might occur with the ageing of the leaf or as a result of a sudden drop in temperature." Simmonds noted that the time taken for the streak to pass into the brown stage is not so definitely related to seasonal temperature. This period was usually relatively short compared with the incubation period. He did, however, suggest that the transition from the streak to the brown stage is stimulated by a sudden fall in temperature.

In Jamaica, where winter temperatures are above those recorded in South Queensland and the range in temperatures is far less, Leach (1946) found that temperature alone had no effect on development but that "fast growing plants developed streaks and spots significantly faster than slow growing plants, the effect being most marked in the case of the development of the spots." His main finding, however, was that spot development was closely correlated with the number of infections per unit area of leaf surface. He noted that "the degree of latency, inherent in spot development, is closely correlated with certain anatomical features of the leaf and with the number of spots which finally develop on a leaf." The results of his work "definitely supported the hypothesis that the rate of development of streaks and spots is dependent upon the number of spores infecting a unit area of leaf surface, also . . . that spots appear first in those areas where the greatest number of streaks and spots develop and that the percentage of streaks which turn into spots is highest where the number of streaks is greatest." Leach postulated the secretion of some toxin by the hyphae and stated: "The greater the number of stomata that become infected per unit area of leaf, the greater will be the growth of internal hyphae and consequently the greater will be the quantity of toxin produced so that the more stomata infected per unit area the quicker will be the loss of tissue resistance and the faster will be the rate of spot development."

In North Queensland there is a seasonal variation in the rate of development of the leaf spot fungus. The observations on disease incidence made at Kamerunga during 1957 and 1958, using the technique described when discussing the seasonal pattern of disease incidence, have been used to illustrate this seasonal fluctuation (Figure 6).

The various data used were arrived at as follows:

Approximate minimum incubation periods for the infections appearing within the marked areas on each leaf were derived by assuming that the infections originated on the expanding heart-leaf. When the appearance of streaks was first recorded the approximate incubation period was obtained by calculating the time that had elapsed since the particular leaf was in the heart-leaf stage.

The period from streak to spot was determined by using a separate symbol for each fresh batch of streaks which was recorded within any one observation area each week. As streaks changed to spots the time involved was calculated back.

For determining growth rate, leaves were numbered as they appeared and it was known from these observations how many leaves were produced in a given month. These figures were used to calculate the number of days between successive leaves. The reciprocal of this value was termed the growth rate. The figure plotted for each month is the average growth rate of the three plants.

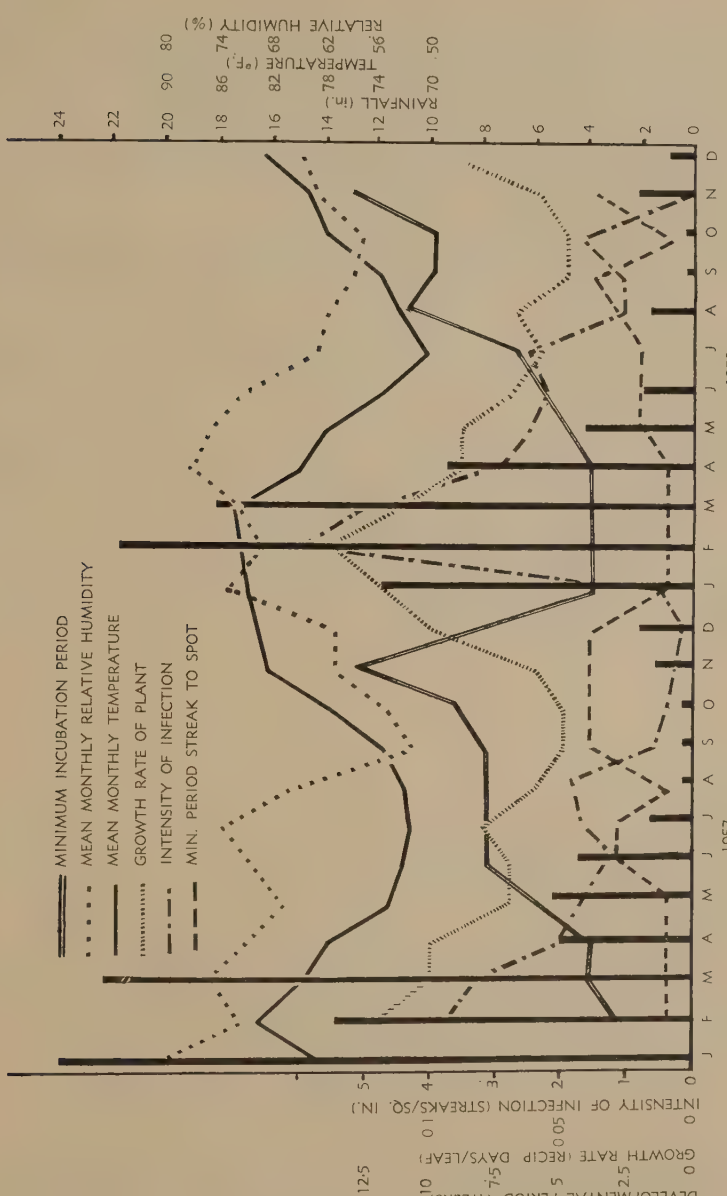
MINIMUM INCUBATION PERIOD
MEAN MONTHLY RELATIVE HUMIDITY
MEAN MONTHLY TEMPERATURE
GROWTH RATE OF PLANT
INTENSITY OF INFECTION
MIN. PERIOD STREAK TO SPOT
DEVELOPMENTAL PERIOD (WEEKS)
GROWTH RATE (RECIP DAYS/LEAF)
INTENSITY OF INFECTION (STREAKS/SQ. IN.)
RAINFALL (in.)
TEMPERATURE (°F.)
RELATIVE HUMIDITY (%)
J F M A M J J A S O N D J F M A M J J A S O N D
1957
1958

Fig. 6.—Seasonal variation in rate of development of leaf spot. Kamerunga, 1957-58.

Intensity of infection is expressed as streaks per sq. in. and the values were determined by the method described on page 217. The arrangement of those values into monthly groups was accomplished by assigning to each month those values for areas in which streaks first became evident during that month. The monthly figures plotted in Figure 6 comprise the minimum incubation period from each monthly group together with the associated intensity of infection and minimum period from streak to sporing spot.

To facilitate the plotting all developmental periods which were originally recorded in days have been expressed as weeks by using the following conversion scale: 0–10 days, 1 week; 11–17 days, 2 weeks; 18–24 days, 3 weeks; and so on.

It is not intended that the times quoted for incubation and streak to spot be construed as accurate estimates of their duration but rather that they be regarded as indices of rate of development.

The values given for humidity are the averages of the mean monthly 9 a.m. and 3 p.m. relative humidities. The total rainfall for each month is plotted.

These observations on natural infection as well as the results of some inoculation experiments (Table 4) are used below to investigate the effect of environment, intensity of infection, growth rate and age of leaf on leaf spot development. The periods covering incubation and the change from streak to mature spot are considered separately.

(e) Influence of Specific Factors on the Incubation Period

(i) Moisture

From Figure 6 it will be seen that in both 1957 and 1958 the combined effect of high rainfall and high daytime humidity was apparent in the short incubation periods for those infections which appeared during January, February, March and April. In 1957 lower rainfall and reduced humidity in April could have been responsible for the lengthening of the incubation period for infections which appeared in May and June, The mean humidity rose again in June and July and this was associated with the flattening of the incubation curve, which extended to include infections appearing during September. The drop in humidity and scant rainfall in August, September and October can be related to the peak in the incubation curve in November.

In 1958 humidity fell steadily from May through to July and rainfall was negligible in July. This was apparently responsible for the sharp rise in incubation for infections which appeared in August. The flattening of the incubation curve for infections appearing during September and October was related to the rain group in August, and the peak in incubation during November was apparently due to the low humidities and negligible rainfall during September and October.

TABLE 4

Developmental Data from Artificial Inoculation Experiments and the Associated Environmental Conditions

Plant No.	Date Inoculated	Date Streaks Appeared	Growth Rate	Intensity of Infection (streaks/sq. in.)	Developmental Periods (days)		Mean Daily Temperature (°F)		Mean Daily Relative Humidity (%)		Rainfall† and Date
					Incubation	Streak to spot	Incubation	Streak to spot	Incubation	Streak to spot	
11	6 [illegible]	2. iii. 1959	·14	58	24–31 (23·5)	7–21	74–96 (85)	74–86 (80)	79	87	Rgs. 13/2–16/2, 3·23 in.; 24/2–2/3, 9·46 in.
12	[illegible]	18. v. 1959	·08	3	40–70 (55)	6–60	69–84 (76·5)	62–82 (72)	78·5	77·5	0·09 in. on 3 wet days to 14/4 ; Rg. 14/4–16/4, 0·95 in.
13	1. v. 1959	9. vi. 1959	·07	29	39–59 (49)	5–27	64–79 (71·5)	61–80 (70·5)	80·5	76·2	0·02 in. on 1/5 ; Rgs. 5/5–18/5, 4·19 in. ; 20/5–28/5, 1·29 in. ; 1/6–8/6, 1·23 in.
14	1. v. 1959	17. vi. 1959	·07	15	47–66 (56·5)	5–33	65–79 (72)	63–79 (71)	79·5	76·2	As Plant 13
15	1. v. 1959	17. vi. 1959	·07	15	47–66 (56·5)	5–33	65–79 (72)	63–79 (71)	79·5	76·2	As Plant 13, plus 11/6–16/6, 0·27 in.
6*	25. vi. 1958	28. viii. 1958	·06	10	64–120 (92)	7–70	59–85 (72)	65–88 (76·5)	68·75	65·75	0·23 in. on 3 wet days to 2/7 ; Nil 2/7–10/8 ; Rg. 11/8–18/8, 1·58 in.
7	25. vi. 1958	30. vii. 1958	·07	56	35–56 (45·5)	7–30	61–83 (72)	62–83 (72·5)	68·5	69	0·23 in. on 3 wet days to 2/7
1	18. vii. 1955	24. viii. 1955	·06	20	37–70 (53·5)	7–44	62–83 (72·5)	65–83 (74)	73·75	74·2	0·16 in. on 5 wet days
2	25. vii. 1955	31. viii. 1955	Flowered	22	36–60 (48)	10–31	62–83 (72·5)	64–83 (74)	73·25	75·5	0·11 in. on 4 wet days to 26/8 ; Rg. 29/8–31/8, 0·71 in.
8	31. vii. 1958	4. ix. 1958	·06	40	35–76 (55·5)	7–42	59–85 (72)	66–89 (77·5)	68·75	67·75	Rg. 11/8–18/8, 1·58in.
9	31. vii. 1958	28. viii. 1958	·1	36	28–68 (48)	7–42	63–84 (73·5)	64–86 (75)	69·0	66·5	Rg. 11/8–18/8, 1·58in.
3	29. viii. 1955	7. x. 1955	·07	23	39–71 (55)	7–39	64–85 (74·5)	70–91 (80·5)	74·75	74·25	Rg. 29/8–5/9, 2·02 in.; 12/9–16/9, 0·97 in.; 19/9–22/9, 0·53 in.; 5/10–7/10, 0·38 in.
10	2. ix. 1958	9. x. 1958	·06	34	37–51 (44)	7–21	65–86 (75·5)	67–89 (78)	64	68·5	25/9–26/9, 0·35 in. ; 2/10, 0·09 in.
4	13. ix. 1955	20. x. 1955	·07	12	37–56 (46·5)	7–34	65–86 (75·5)	70–91 (80·5)	75·5	72·75	Rg. 13/9–22/9, 1·36 in. ; 5/10–10/10, 0·43 in.
5	14. xii. 1955	16. i. 1956	·09	10	33–63 (48)	7–35	74–95 (84·5)	74–91 (82·5)	77	80·25	16/12, 0·03 in. ; 19/12, 0·80 in. Rg. 8/1–13/1, 3·40 in.

* Plant 6 grown in shelter of lath-house.

† Rg. = Rain group.

Another illustration of the effect of moisture on the incubation period is provided by a comparison between Plants 6 and 7 in the inoculation experiments summarized in Table 4. These plants were inoculated on the same day from the same stock spore suspension. Plant 6 (minimum incubation period 64 days) was grown in the shelter of a lath-house, while Plant 7 (minimum incubation period 35 days) was in the open and exposed to dews, which were very frequent at that time of the year. However, as mentioned previously, this evidence is not positive because of the possibility of some metabolic disturbance to the plant due to the partial shading. An additional illustration of the effect of rainfall on intensity of infection will be found in Figure 7.

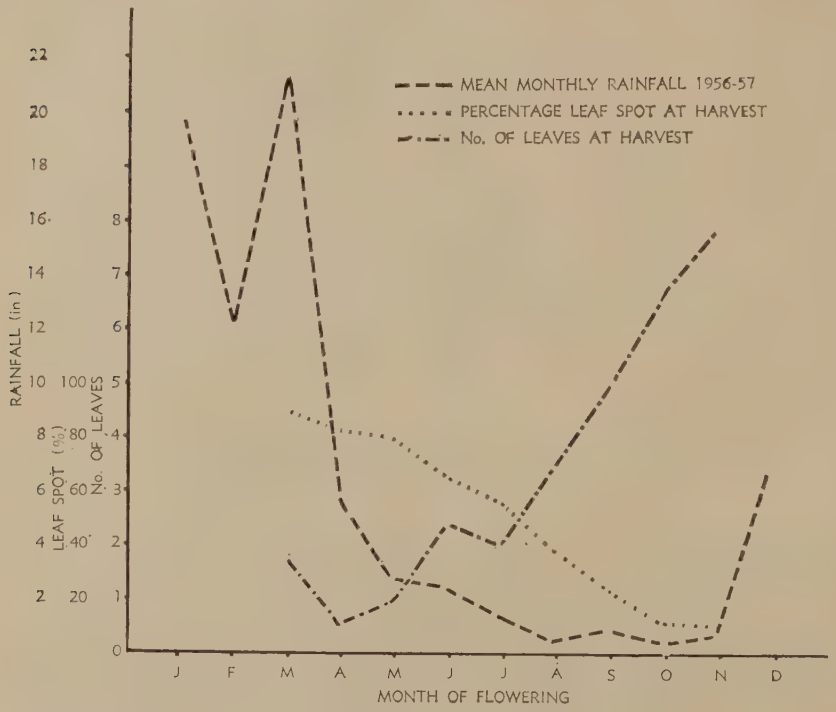

Fig. 7.—Effect of time of flowering on leaf spot percentage at harvest. Combined data from unsprayed plants in Trial 5 and 6. Stoney Creek, 1956 and 1957.

(ii) Temperature

The variation in minimum incubation period (Figure 6) can be related to temperature as well as to moisture. In both 1957 and 1958 incubation period lengthened as temperature decreased. The maximum incubation period in each year was recorded for those few streaks which appeared in November from infections which apparently originated some three months earlier in August at a time when the daily minimum temperature was reaching very low levels. December

infection is presumed to have originated in October and the higher minimum temperatures recorded in this month and November were associated with a marked reduction in the incubation period.

(iii) Intensity of Infection

In Figure 6 the minimum incubation period is inversely proportional to intensity of infection, which was highest during the wet season and lowest at the dry season in both years. Intensity of infection itself broadly follows the growth rate and both are probably related to the same general meteorological features.

When the incubation periods for the artificial inoculations (Table 4) on Plants 8 and 9 (intensity of infection 36-40 streaks/sq. in., minimum incubation period 4-5 weeks) are compared with those calculated for natural infections which appeared during the same period of the year (August intensity of infection 1·2–1·8 streaks/sq. in., minimum incubation period 8–11 weeks), the reduction in incubation associated with higher intensities of infection is quite apparent.

The difference in incubation period for the inoculations on Plants 13 (intensity of infection 29 streaks/sq. in., minimum incubation period 39 days) and 14 or 15 (intensity of infection 15 streaks/sq. in., minimum incubation period 47 days) also points to a relationship between these two factors.

Further evidence is furnished in Table 5, which summarizes the results of the inoculations on Plant 13 in April 1959.

TABLE 5

Effect of Intensity of Infection on Development (Artificial Inoculations Plant 13, 8. iv. 1959)

Intensity of Infection (streaks/sq. in.)	Developmental Period (Days)	
	Incubation	Streak to Sporing Spot
9·6	40–48	5–60
9·2	40–61	9–30
7·6	40–70	5–27
2·8	53–70	5–30
1·2	53–70	19–37
0·4	70	19

(iv) Growth Rate

In Figure 6 it can be seen that incubation is least for those infections which occurred when growth rate was highest. Conversely, it is at a maximum for those infections that took place when the plants were growing very slowly. Growth rate itself appears to be closely related to both temperature and rainfall. In the field, therefore, any association of growth rate with rate of development of leaf spot would be secondary, the primary influences being temperature and rainfall.

A further indication appears in the inoculation experiments (Table 4). Plant 9 (growth rate 0·1, minimum incubation period 28 days) was a sucker from the same stool as Plant 8 (growth rate 0·06, minimum incubation period 35 days). They were inoculated on the same day from the same stock spore suspension. The shorter incubation period is apparently related to the higher growth rate for the intensities of infection were very similar.

(v) Age of Leaf

When the results of various inoculation experiments were examined to provide information on the effect of age of leaf on rate of spot development, the data presented in Table 6 were obtained. It can be seen that there is no indication of any variation in incubation period when rate of development on the youngest leaves is compared with that on leaves as old as the seventh. However, it must be admitted that the seventh leaf on a banana plant is, to the eye at least, far from being senescent. This evidence fails to support the suggestion that the long incubation period following heart-leaf inoculation is due to some essential maturation changes in the leaf itself.

Before concluding this section it should be mentioned that maximum periods of incubation as long as 22 weeks have been calculated for some of the dry season infections recorded in 1957 and 1958 at Kamerunga using the method described above and assuming that the infection processes were initiated on the unfolding heart-leaf. Incubation periods of this duration are obviously apparent rather than real.

The factors which are involved in the prolongation of the apparent period of incubation are infection of leaves when they are older than the heart-leaf stage and dormancy of the spores.

The latter has been demonstrated experimentally in North Queensland. Microscopic examination of banana foliage inoculated with conidia during the dry season has shown that these spores may remain dormant on the leaf surface for a considerable time before the infection processes are initiated. In addition, there can be a considerable variation in the rate of germination of spores from the same suspension applied to the same leaf and exposed to the same environment. The reason for this variation is not obvious.

Simmonds (1939) quoted strong circumstantial evidence to indicate that in South Queensland conidia of *M. musicola* remained ungerminated on the leaf surface for periods up to 22 days in the absence of "effectual" rain.

(f) Factors Influencing the Change from Streak to Sporing Spot

(i) Moisture

It is clear from Figure 6 that the minimum period streak to spot was least during the wet halves of both years. The steep drop in this curve in January

TABLE 6

Effect of Age of Leaf on Rate of Leaf Spot Development (in weeks)

Leaf Position When Inoculated (Numbered from top down)	1		2		3		4		5		6		7	
Date Inoculated	Incubation	Streak to Spot	Incubation	Streak to Spot	Incubation	Streak to Spot	Incubation	Streak to Spot	Incubation	Streak to Spot	Incubation	Streak to Spot	Incubation	Streak to Spot
18. vii. 1955	5– 9	1–4	5–10	1–4	5–10	1–6	5–7	1–5	6–7	2–5	..	..	..	..
18. vii. 1955	5– 8	2–4	5– 8	2–4	5–10	2–4	5–8	2–5	..	..	..	..	..	..
29. viii. 1955	6– 9	1–4	6– 9	1–5	6– 9	3–6	6–9	1–4	6–9	2–5	6–9	2–5	6	1–2
13. ix. 1955	5– 8	2–4	5– 8	1–5	5– 8	2–5	6–9	1–4	5–9	1–5	..	..	..	..
14. xii. 1955	5– 8	1–4	5– 9	1–4	5– 7	1–3	5–8	1–3	5–8	1–3	..	..	..	..
25. vi. 1958	9–17	1–8	9–17	1–6	9–12	2–5	..	..	..	..	..	..	..	..
25. vi. 1958	5– 8	1–4	5– 8	1–4	5– 8	1–4	..	..	..	..	..	..	..	..
31. vii. 1958	4–11	1–6	4–10	1–4	4–11	1–4	..	..	..	..	..	..	..	..
31. vii. 1958	5–12	1–6	5–11	1–6	5–10	1–5	..	..	..	..	..	..	..	..
8. iv. 1959	8–10	3–5	6– 9	1–9	8–10	1–4	6–10	1–6	..	..	..	..	..	..
1. v. 1959	7–10	1–5	7– 9	1–4	7– 9	1–3	7–9	1–3	7–9	1–3	..	..	..	..
1. v. 1959	7–10	1–5	7–10	1–5	7– 9	1–5	7–9	1–3	7–9	1–3	..	..	..	..
1. v. 1959	6–10	1–4	6– 9	1–3	6– 8	1–9	..	..	..	..	..	..	..	..

1958 could be associated with the higher humidities and rainfall during this month. However, there is no evidence in Table 4 to indicate any relation between the duration of this stage of development and atmospheric moisture.

(ii) Temperature

A direct relationship between temperature and period streak to sporing spot is not obvious from an examination of either Figure 6 or Table 4. The minimum occurs in the months of January to May with high to moderate temperatures but this is more obviously related to heavy rainfall of these months. The maximum occurs during the drier months with low to moderate temperatures.

(iii) Intensity of Infection

The seasonal variation in minimum period streak to spot illustrated in Figure 6 can be more closely correlated with intensity of infection than with any other factor. The reduced values in August 1957 and October 1958 can be related to the increased intensities in those months. When the results of the inoculation experiments (Table 4) are compared with the field observations it can be seen that the much higher intensities of infection resulting from the inoculations have exerted a marked influence on the duration of this period of development, for the highest minimum value recorded is 10 days. The effect of reduced intensity of infection in lengthening the time taken for streaks to change to sporing spots is also illustrated in Table 5. The apparent relationship between the period under discussion and high rainfall could well be due to the effect of the latter on intensity of infection.

(iv) Growth Rate

The relationship between period streak to spot and growth rate, if any, is of an inverse nature as in the case of incubation period. This probably indicates a general link with the principal meterological features.

(g) Discussion

In summing up the implications discussed above, it is evident that atmospheric moisture, temperature, intensity of infection and growth rate of the plant may influence the duration of incubation, i.e. of latency. The latent period during which infection is present but not obvious can be subdivided into the period when the fungus is epiphyllic, from germination of the spore to penetration of the leaf by the infection hypha, and the endogenous period which precedes the appearance of the streak stage lesion.

Moisture and temperature, the effect of which on germination and germ-tube growth has already been discussed, would, undoubtedly, be the decisive influence on development until the fungus reaches the inside of the leaf. Once penetration has taken place the interaction of temperature, intensity of infection and perhaps the growth rate of the plant have to be considered.

The duration of the period from streak to sporing spot is largely dependent on the number of infections per unit leaf area and no effect of environment is clearly indicated in this case.

In this experimental work it was noted that the first streaks to appear are not necessarily the first to change to spots; although usually if a large number of streaks were recorded in the first batch in a particular observation area, some of these would be included among the first to change to spots. The streaks which changed in the shortest time were very often those which appeared last. This could be interpreted as meaning that the spores from which these late streaks arose germinated later so that penetration took place later, by which time the earlier infections had rendered the substrate less resistant or more suitable for development.

As stated above, Leach (1946) considered that increased production of toxin by the fungus and loss of resistance of the leaf tissue was responsible for the acceleration in rate of development of leaf spot with increase in intensity of infection.

It can also be regarded as being due to an increase in the intensity of enzymatic reactions due to increased concentration of the enzymes released by the fungus consequent on the increased amount of mycelium present. By the same reasoning the effect of temperature can be regarded as an effect on reaction rate of the enzymes as well as on the rate of respiration and vegetative growth of the fungus.

Similarly, the effect of increased growth rate of the plant, i.e. increased plant vigour, can be credited to an increased reaction rate consequent on increased hydration and increased concentration of the substrate, two factors which increase the rate of enzymatic reactions (Meyer and Anderson 1956).

Other variations in composition between the leaves of a slowly and a quickly developing plant could alter the situation through the enzyme complex or fungal nutrition.

The lack of evidence of any effect of growth rate on the change from streak to spot could be attributed to the fact that at this stage in leaf spot development enzyme activity becomes very intense, as evidenced by the rapid infiltration of the tissue surrounding the streak which precedes the actual necrosis. This high concentration is evidently sufficient to overcome the normal host resistance which is adequate to restrain the fungus in the earlier stages.

IV. CONTROL EXPERIMENTS IN NORTH QUEENSLAND

(a) Historical Summary

Work on the control of banana leaf spot was carried out in South Queensland during the years 1928–1930. Simmonds (1933) investigated the effect of various practices, including dusting with copper fungicides and stripping of infected

foliage. No definite conclusions were derived from these experiments and it was considered doubtful whether the control exercised by the standard fungicides then available was sufficient to justify regular treatment under South Queensland conditions.

In 1937 it was reported from Surinam that spraying plantations two or three times a year with Bordeaux mixture or Bayer fungicide had given good results (Stahel 1937).

In New South Wales it was demonstrated that summer treatment of plantations with fungicides gave promising results and that excellent control was obtained by applying Bordeaux (4-4-40) each month from mid-December to mid-April (Magee and Foster 1938).

A study of the influence of weather on leaf spot development made by Simmonds in South Queensland about this time also stressed the importance of summer infections and enabled recommendations to be made regarding the timing of sprays in a control programme (Simmonds 1939).

Meanwhile the spread of the disease in the Caribbean region led to investigations into leaf spot control in Jamaica. Here the seasonal effect of the disease was less marked and spraying had to be continued throughout the year. It was found that spraying with "Perenox" (¼–½ per cent.) at intervals of either 2 or 3 weeks using 100-200 gal per ac gave promising results. Bordeaux mixture (4–4–40) also gave control but the cuprous oxide preparation was superior (Ward 1938). Later work in Jamaica demonstrated the importance of the ascigerous stage of the leaf spot fungus and showed that spraying effectively controlled infection by conidia but not by ascospores (Leach 1946).

In the large banana plantations in Central America, where the disease was well established by the early 1940s, the threat to production was met by the adoption of a costly control programme. Leaf spot was controlled by spraying in cycles of 2–3 weeks with copper sprays, employing stationary spraying apparatus. The mixture was delivered from central pumping stations through permanent pipelines reticulated throughout the plantations. From these the spray was distributed by hoses (Wardlaw 1941).

Continued investigations into the control of leaf spot and speckle in New South Wales resulted in the adoption of control measures consisting of spray applications commencing in early December and repeated each month until March. The mixture recommended was copper oxychloride (1½ lb to 40 gal) and colloidal sulphur (1 lb to 40 gal) plus a wetting agent. It was stressed that the spray should be directed at the heart-leaf and at the under-sides of the three youngest fully opened leaves (Magee 1945). Application was achieved by the use of portable power pumps or by the installation of permanent pipelines radiating from a central pumping station. For various reasons only a limited number of growers adopted these control measures.

More recent investigators in Jamaica gave attention to improving the performance of leaf spot fungicides by the use of better wetting agents and it was found that the use of a new wetter, sodium succinate, with copper carbonate, Bordeaux mixture or "Perenox" gave good control (Anon. 1953, 1954). The benefits of using suitable wetting agents with banana leaf spot sprays have since been confirmed at Long Ashton (Kearns and Martin 1957).

In 1953 an important development in leaf spot control took place in Guadeloupe. French workers began experimenting with low-volume techniques and a radical departure from conventional low-volume methods was made by the substitution of mineral oil for water as the carrier for the fungicide.

In the initial experiments it was found that fogging with zineb or copper oxychloride, both suspended in oil, gave better leaf spot control than the customary copper oxychloride spray (Guyot 1953). Guyot later showed that two thermo-aerosol treatments in five months were more efficient than fortnightly knapsack spraying with aqueous zineb/copper oxychloride suspensions during the same period. He attributed this result to the fortuitous timing of the fog applications. It was also demonstrated that a fine oily mist applied with a "Minimicron" shoulder-mounted mister gave better control than did a fog applied with a "Swingfog" (Cuillé and Guyot 1954).

When misting and fogging were again compared it was found that misting with zineb/oil was superior to fogging with the same mixture when the output was the same, i.e. 2 gal per ac. Misting with zineb/oil was superior to misting with oil alone but the beneficial effect of the oil alone treatment was undeniable (Guyot and Cuillé 1955).

The low-volume treatment with oily mixtures was quickly adopted elsewhere and has been responsible for a very high degree of leaf spot control. In the Cameroons a widespread outbreak of Cercospora leaf spot was arrested by misting with copper/oil mixture applied either as a mist by knapsack misters or by high-altitude "fogging" from fixed-wing light aircraft. The mixture was applied at intervals of either 2 weeks or 3 weeks (Merle, Cuillé, and De Laroussilhe 1958).

Phytotoxicity from continued application of oil is proving to be a problem, however, and it appears that this treatment can cause premature death of foliage and a reduction in fruit weight (Anon. 1957). In addition, it has been reported that continued use of oil causes a reduction in plant growth and fruit yield (W. A. Brun, personal communication August 1959). F. J. D. Thomas (personal communication July 1959) stated: "It is said that the liability of the banana stem to bark or sheath splitting is increased where oil is used. Continuation with oil spraying, during periods of drought, or in plantations where root efficiency is weakened due to eelworm attack, makes the plants turn yellow and take on a distinctly unhealthy appearance."

The low-volume technique has been used also for the application of a water-based fungicide for leaf spot control. In Ecuador good control of the disease was obtained by misting bananas with Banacobre-Sandoz (a cuprous oxide containing a special waxy sticker) suspended in water. The output was 10 gal per ac and the treatments were repeated at intervals of 3–4 weeks (Tollenaar 1955).

It has been reported from that country that the control of leaf spot obtained by low-volume spraying was equal to or better than that obtained with earlier methods. At 3 gal per ac, "agricultural oil" in water with various fungicides gave good control of the disease and results improved as the oil was increased from 20 per cent. to 60 per cent. "Agricultural oil" alone at 3 gal per ac was almost equally good (Desrosiers and Ampuero 1957).

While the copper fungicides and zineb appear to be the only compounds which have been used in large-scale leaf spot control experiments, many others have been tested (Merny 1954, 1955). Merny, in his first tests of eight fungicides, found that only zineb was better than copper oxychloride. He later tested another 24 compounds for toxicity against *Cercospora musae,* using copper oxychloride as a standard. Copper oxide and zinc coposil (both in oil) were superior to copper oxychloride.

(b) High-volume Spraying with Water-based Fungicides

North Queensland banana plantations are either planted on arable land or are accessible to motor vehicles and hence are suitable for spray application by means of high-pressure spray outfits. Water supply is no problem in the coastal areas, where there is an abundance of fresh running water handy to most plantations at all times of the year. For this reason the main purpose of the work reported here has been an investigation of control measures using high-volume methods.

In the high-volume experiments a series of six major spray trials was carried out during the years 1951-1958. Trials 1 and 2 were located at Mission Beach, near Tully; Trials 3 to 6 on a plantation at Stoney Creek, near Cairns.

Trials 1 to 4 were designed to investigate the economics of protectant spraying for leaf spot control with the standard fungicides available at that time. In the discussion which follows these trials are considered conjointly and the results summarized.

An important development was evident in the results of Trials 5 and 6, viz. the improvement in leaf spot control due to the suppressive effect of mixtures containing white oil emulsion, and these two trials are therefore considered separately in more detail.

(i) Procedure

In all these trials the variety sprayed was either Cavendish or its tall mutant Mons Mari. The work was carried out with a small spray plant consisting

of a twin-cylinder pump powered by a 98 c.c. 4-stroke engine. Working pressures of up to 250 lb per sq. in. were used. Delivery was by high-pressure spray hosing through an adjustable swirling-type nozzle. Under normal working conditions the spray plant was transported through the plantations on a motor truck.

Output per acre varied considerably according to size of plant, planting distance and method of spraying used. An average dosage for a heart-leaf spray on a plant nearing flowering was half-a-gallon. A cover spray required considerably more.

In this work heart-leaf spraying was regarded as treatment of the unfolding heart-leaf and all the leaves which had emerged since the previous spray round, the spray being directed particularly at the lower surfaces of the leaves. Cover spraying was regarded as treatment of all the leaves on the plant on both upper and lower leaf surfaces.

In all experiments a randomized block layout was used. Single-row plots were employed and the number of plants used varied from 7 to 10 per plot in individual experiments.

The assessment of results was achieved in the following manner:

(1) *Infection.*—When a plant flowered and again when its bunch was harvested a count was made of live leaves and each leaf was given a percentage rating for leaf spot infection based on a visual estimate of the damage. From these data an average figure for amount of infection per leaf per plant at each of these critical stages was calculated.

(2) *Yield.*—In the Mission Beach experiments the bunches were weighed when they were cut each week. The yield data were then expressed as a mean weight of marketable fruit per plant. In arriving at this figure the grower's practice of discarding any bunches which ripened prematurely was followed.

In the experiments at Stoney Creek the bunches were harvested once or twice each week. Each bunch was weighed immediately it was cut. If a bunch carried any prematurely ripened fingers the weight of the fruit thus involved was estimated and expressed as fruit wastage. In addition, three fingers were chosen at random on the third hand of each bunch. The length (L) and girth (G) of each of these fingers was measured (in in.). As a convenient index of fruit size the quantity $L \times G^2$, which has the dimensions of volume, was used.

Butt circumference is not regarded in this work as being related to spray treatment but rather to plant vigour. Butt circumference measurements were made in these experiments in order to make allowance for the large variations in stool vigour which are usually evident in a banana plantation. Bunch size in relation to number of fruit appeared to be proportional to butt circumference, and bunch weight is dependent on bunch size as well as on other factors such as fruit size which definitely may be related to spray treatment.

The yields were then assessed on adjusted bunch weights, which were estimates of the bunch weights adjusted by co-variance analysis for variations in butt circumference.

(3) *Economic Returns.*—Plant density in the Stoney Creek trials was approximately 500 stools per acre. The prices which the grower received for fruit harvested from the experimental blocks during the period of the experiments ranged from 6d. to 1s. 3d. per lb. For the purposes of this experimental work a figure for estimated gross return per plant due to spraying was arrived at by allowing a price of 7d. per lb (£2 10s. per standard banana case). This was calculated for those adjusted mean bunch weights which were significantly greater than the mean bunch weight for the unsprayed treatment. The gross return per acre was then assessed on the basis of a harvest of 500 bunches per acre.

(ii) Trials 1 to 4, 1951–1955

In these experiments copper oxychloride was compared with copper oxychloride plus wettable sulphur. Copper oxychloride, Bordeaux mixture (3-2-40) and home-made cuprous oxide (Mandelson 1933) were compared with the organic fungicides thiram, ziram and zineb. The only additive used was a wetting agent, which was varied in the different experiments. "Lissapol N300" (0·1 per cent.), "Comprox" (0·3 per cent.) and soap powder (1½ lb-40 gal) were all tried. Soap powder was incompatible with Bordeaux mixture.

In addition, various schedules were tested, the chief purpose being to determine the minimum number of heart-leaf sprays which was necessary to provide satisfactory control under North Queensland conditions. A schedule involving cover spraying commencing late and carried through until the bunch was harvested was also investigated.

The information gained from this phase of the work can be summarized as follows:

(1) Copper oxychloride and copper oxychloride plus wettable sulphur were equal in efficiency.

(2) The copper fungicides as a group were superior to the organic fungicides, and copper oxychloride (1½ lb-40 gal) and Bordeaux mixture (3-2-40) were more efficient than home-made cuprous oxide (3 gal-40 gal).

(3) The effects of leaf spot spraying were cumulative and progressive reduction in inoculum potential resulted in much better control in the second crop when two successive ratoons were treated with the same materials.

(4) The control of the disease obtained with a schedule of heart-leaf sprays at intervals of four weeks which commenced prior to the wet season (November) and continued until the plants flowered was equal to that which resulted from a similar schedule which commenced some months earlier (July). However, some

degree of control was obtained by a schedule in which heart-leaf spraying was commenced in March and was repeated at intervals of four weeks until the plants flowered. The importance of fungicidal cover during the late summer and autumn was thus emphasized.

(5) An interesting point was that some protection was given by late cover sprays which were first applied when the plants were close to flowering and were continued after the bunches were thrown. This indicated that fresh infection during the period between flowering and harvest could be an important cause of damage.

(6) No significant differences either in bunch weight or in amount of fruit wastage due to premature ripening resulted from the use of brown paper or hessian bunch covers.

(7) Increments in gross monetary return per acre of up to £187 10s. were estimated for the best treatments, which were copper oxychloride (1½ lb-40 gal) and Bordeaux mixture (3-2-40).

(iii) Trial 5, 1956–1957

This experiment was designed to determine whether the efficacy of a leaf spot fungicide, in this case Bordeaux mixture (3-2-40), could be improved by the use of a sticker. White oil (1-160) was chosen because this adhesive had given good results when used with Bordeaux mixture for citrus black spot control in New South Wales (Kiely 1950) and also when used with cuprous oxide mixture for the control of the same disease in North Queensland. This is the common agricultural white oil prepared for use as an insecticide. The second sticker used was "Calspred", a proprietary product containing calcium caseinate. In addition, in an attempt to improve the antisporulant properties of the mixture, malachite green, which has been mentioned as a possible antisporulant (Horsfall 1945), was included. Urea was likewise used as an additive, mainly because it had been suggested to the author as a depressor of sporulation (G. D. Bowen, personal communication 1955).

Treatments were as follows:

A. Bordeaux mixture (3-2-40) plus "Calspred".

B. Bordeaux mixture (3-2-40) plus urea (1 per cent.) plus glucose (·25 per cent.) plus wetting agent ("Lissapol N300" 0·03 per cent.).

C. Bordeaux mixture (3-2-40) plus wetting agent ("Lissapol N300" 0·03 per cent.).

D. Bordeaux mixture (3-2-40) plus white oil (1-160) plus malachite green (1-10,000).

E1. Unsprayed. Bagged.

E2. Unsprayed.

All bunches on sprayed plants were bagged (enclosed in brown paper or hessian covers). Two schedules were used:

Schedule 1.—Cover spray in January followed by heart-leaf sprays repeated at intervals of four weeks until the plants flowered.

Schedule 2.—Late cover sprays commenced in May and repeated at intervals of four weeks until the bunches were reaped.

The results are summarized in Tables 7, 8, 9 and 10.

TABLE 7

Mean Values for Number of Leaves at Flowering and Harvest, Trial 5, 1956–1957

	Flowering		Harvest	
	1 Heart-leaf	2 Cover	1 Heart-leaf	2 Cover
A. Bordeaux mixture + "Calspred" ..	11·0	10·0	5·3	4·6
B. Bordeaux mixture + Urea	11·2	10·1	6·0	4·0
C. Bordeaux mixture + "Lissapol N300"	11·0	10·0	6·4	4·6
D. Bordeaux mixture + White oil + Malachite green	13·4	10·7	6·7	5·4
E1. Unsprayed. Bagged	9·0	..	1·9	..
E2. Unsprayed. Not bagged	9·7	..	3·6	..
	s.e. = ·38	..	s.e. = ·44	..
Necessary differences for significance 5%	1·1		1·3	
Necessary differences for significance 1%	1·5		1·7	
	D1 >> all others B1, C1, A1, D2 >> E1 B1 > A2, B2, C2, E2 C1, A1 > E2 B2 > E1		D1, C1 >> A2, B2, C2, E1, E2 B1 >> B2, E1, E2 D2 >> E1, E2 A1, A2, C2, B2 >> E1 E2 > E1 D1 > A1 B1 > A2, C2 D2 > B2 A1 > E2	

TABLE 8

Leaf Spot Infection—Equivalent Percentages, Trial 5, 1956–1957

	Flowering		Harvest	
	1 Heart-leaf	2 Cover	1 Heart-leaf	2 Cover
A. Bordeaux mixture + "Calspred" ..	14·0	15·0	18·7	26·4
B. Bordeaux mixture + Urea	15·0	14·4	22·2	21·6
C. Bordeaux mixture + "Lissapol N300"	16·3	14·4	20·1	21·5
D. Bordeaux mixture + White oil + Malachite green	9·1	13·5	11·0	18·7
E1. Unsprayed. Bagged	17·7	..	76·4	..
E2. Unsprayed. Not bagged	14·2	..	40·6	..
	No significant differences		All others << E1 D1 << E2	

TABLE 9

Mean Values for Bunch Weights and Butt Circumferences, Trial 5, 1956–1957

	Observed Bunch Weight		Butt Circumference		Adjusted Bunch Weight		Estimated Increased Gross Return (per plant)	
	1 Heart-leaf	2 Cover	1 Heart-leaf	2 Cover	1 Heart-leaf	2 Cover	1 Heart-leaf	2 Cover
							s. d.	*s. d.*
A. Bordeaux mixture + "Calspred" ..	35·7	37·0	24·1	24·9	37·7	35·9	2 10	1 9
B. Bordeaux mixture + Urea	36·8	34·1	24·2	23·8	38·6	37·1	3 4	2 5
C. Bordeaux mixture + "Lissapol N300"	42·0	36·0	25·8	24·3	37·6	37·3	2 9	2 6
D. Bordeaux mixture + White oil + Malachite green	40·1	36·7	24·3	24·8	41·3	36·3	4 11	2 0
E1. Unsprayed. Bagged	30·4	..	24·7	..	30·2	..	..	..
E2. Unsprayed. Not bagged	36·0	..	25·4	..	32·9	..	..	..
	s.e. = 2·45				s.e. = 1·52			
	No significant differences				D1 >> E1, E2 A1, B1, C1, C2, B2, D2 >> E1 A2 > E1 D1 > A2, D2 B1, A1, C1 > E2			

TABLE 10

Mean Values for Fruit Size ($L \times G^2$), Trial 5, 1956–1957

	1 Heart-leaf	2 Cover
A. Bordeaux mixture + "Calspred"	199·8	184·7
B. Bordeaux mixture + Urea	201·1	176·6
C. Bordeaux mixture + "Lissapol N300"	211·8	189·5
D. Bordeaux mixture + White oil + Malachite green	208·8	188·5
E1. Unsprayed. Bagged	154·2	..
E2. Unsprayed. Not bagged	183·5	..
	s.e. = 8·71	
	C1, D1, B1, A1, C2, D2 >> E1 C1 >> B2 C1 > A2, E2 D1 > B2 A2, E2 > E1	

When the cover sprays (Schedule 2) commenced in May all plants in these plots except a very few backward stools were treated. Spraying was initiated on these odd plants when they reached an appropriate size. Some of the Schedule 2 plants had flowered when they received their first treatment in May but the majority were still producing leaves.

It is apparent from the results that the outstanding feature of this experiment was the success of Bordeaux mixture plus white oil plus malachite green, Schedule 1. The efficacy of this mixture was also evident when it was used as a late cover spray.

Observations made during the course of this trial showed that the increased efficacy of Bordeaux mixture (3-2-40) when it was used with malachite green and white oil was not due to any superior sticking qualities but to the fact that it suppressed or delayed leaf spot development, i.e. it was a potent fungistatic agent. This phenomenon will be discussed in detail in a later section of this article.

There was little difference between the other treatments. The slight differences in performance between Treatments B (Bordeaux mixture/urea/"Lissapol N300") and C (Bordeaux mixture/"Lissapol N300") could have been due to the fact that the 0·25 per cent. glucose included in the urea formula brought about a partial reduction of the basic copper sulphate to cuprous oxide. Schedule 2 treatments were consistently inferior to their Schedule 1 counterparts but in most cases were not significantly so.

The estimated increment in gross monetary return per acre ranged from £123 (Bordeaux mixture/white oil/malachite green, Schedule 1) to £43 15s. (Bordeaux mixture/"Calspred", Schedule 2).

The seasonal variation in disease intensity which has been discussed previously had obvious effects on the results of this trial. In Figure 7 leaf counts and leaf spot appraisals made for the 1956 and 1957 trials on the unsprayed plants when their bunches were cut have been related to time of flowering and mean monthly rainfall. There was a definite relationship between the time of flowering and the amount of leaf spot at harvest. Those plants which flowered late in the year exhibited a much reduced amount of leaf spot damage.

The apparently anomalous results in Tables 7 and 10 showing Treatment E1 (unsprayed, bagged) to be significantly worse than E2 (unsprayed, not bagged) are better understood when it is considered that 35 per cent. of the E1 plants flowered in the April-May period compared with 16 per cent. of the E2 plants during the same critical period. Nevertheless, the results indicated that in this experiment bagging bunches on unsprayed plants was of no benefit.

(iv) Trial 6, 1956–1958

Because of the success of the Bordeaux mixture/white oil/malachite green formula, the use of these two additives with other fungicides was investigated. It was also necessary to know whether the increased efficacy gained by the use of white oil plus malachite green in the spray mix could be equalled by the addition of white oil alone.

The stools used in the experiment were first ratoons, the mother plants of which had comprised portion of the experimental block used in Trial 5.

Treatments were as follows:

A. Bordeaux mixture (3-2-40) plus white oil (1-160).

B. Copper oxychloride (1½-40) plus white oil (1-160) plus malachite green (1-10,000).

C. Zineb (1½-40) plus white oil (1-160) plus malachite green (1-10,000).

D. Bordeaux mixture (3-2-40) plus white oil (1-160) plus malachite green (1-10,000).

E. Unsprayed.

Spraying commenced in December 1956, when most plants were spear suckers or suckers with first broad leaves commencing to unfurl. Subsequent applications were heart-leaf sprays repeated at intervals of four weeks until the plants flowered.

TABLE 11

Number of Leaves, Percentage Leaf Spot, and Fruit Size ($L \times G^2$), Trial 6, 1956–1958

—	No. of Leaves		Percentage Leaf Spot (Equiv. %)		Fruit Size
	Flowering	Harvest	Flowering	Harvest	
A. Bordeaux mixture + White oil ..	13·0	7·4	2·4	6·4	218·0
B. Copper oxychloride + White oil + Malachite green	12·8	6·7	1·7	7·8	220·2
C. Zineb + White oil + Malachite green	14·0	8·3	3·4	9·7	224·5
D. Bordeaux mixture + White oil + Malachite green	13·4	7·5	2·7	7·7	216·6
E. Unsprayed	10·0	4·0	14·4	51·2	189·1
s.e.	·41	·43	1·14	3·92	5·62
	A, B, C, D >> E		A, B, C, D << E		A, B, C, D >> E

The results are given in Tables 11 and 12 and illustrated in Figures 8 and 9.

All the spray treatments were significantly better than the controls, and Bordeaux mixture (3-2-40)/white oil was equal in efficacy to Bordeaux (3-2-40)/white oil/malachite green.

The increment in estimated gross monetary return per acre ranged from £122 18s. 4d. (zineb/white oil/malachite green) to £87 10s. (Bordeaux mixture/white oil).

The effect of the dry season on leaf spot incidence in the block was once more particularly noticeable (Figure 7). It was apparent in this experiment that plants which flowered later than July retained sufficient foliage to allow the fruit to reach the desired state of fullness without the benefit of spray treatment.

TABLE 12

Mean Values for Bunch Weight and Butt Circumference, Trial 6, 1956–1958

—	Observed Bunch Weight	Butt Circum-ference	Adjusted Bunch Weight	Estimated Increased Gross Return (per plant)
				s. d.
A. Bordeaux mixture + White oil	46·8	28·5	45·5	3 6
B. Copper oxychloride + White oil + Malachite green	47·0	27·9	47·3	4 8
C. Zineb + White oil + Malachite green	48·4	28·2	47·9	4 11
D. Bordeaux mixture + White oil + Malachite green	43·9	27·1	46·3	4 0
E. Unsprayed	40·2	28·3	39·4	..
s.e.	1·93	..	1·57	..
	A, B, C > E		C, B >> E D, A > E	

Fig. 8.—Appearance at time of harvest of a plant sprayed with zineb + white oil + malachite green. Trial 6, Stoney Creek, July 1957.

Fig. 9.—Appearance at time of harvest of an unsprayed plant. Trial 6, Stoney Creek, July 1957.

(c) Low-volume Treatment with an Oil-based Fungicide

When details of the low-volume technique employing oils and oil/fungicide mixtures developed by Guyot and Cuillé in the Antilles became known a limited amount of work was done on this aspect of the leaf spot problem. This was carried out in the hope that the technique would prove suitable for use in South Queensland. Here the majority of plantations are on hillside sites and motor vehicles cannot be used for transport. Water supplies are either absent or deficient on most farms.

(i) Procedure

The machine used was a "Swingfog SN6." This was the only low-volume machine available in Queensland at the time. This machine could be fitted with an atomizer for misting or a fogging attachment as desired. A 1·1 mm flow control jet was generally used for misting and a 1·0 mm jet for fogging. The mixtures employed were slight modifications of those tested by the French workers in their early trials, namely:

Misting mixture:	Copper oxychloride	6½ lb
	Oil (4½ gal "Vacuum EF 217S"; 1 gal Dieselene)	5½ gal
Fogging mixture:	Copper oxychloride	12 lb
	Oil (2 gal lubricating oil SAE 30; 2 gal Dieselene)	4 gal

The treatments were applied at a slow walking pace. The operator walked along each row, the direction of travel being at right angles to the wind, with the mist or fog discharging in the direction of the wind. The output in the case of misting was 1½-2 gal per ac and for fogging ¾-1 gal per ac.

The method of assessing the results was as follows: The centre row in each plot was regarded as the datum row. Leaf spot damage on each plant in this row was assessed when it flowered by making leaf counts and leaf spot appraisals as in the experiments described above. The same procedure was employed to assess leaf spot damage on the followers when the treatments were terminated in September 1957.

The bunch harvested from each plant in the datum row was weighed and the data were used to calculate an average bunch weight for each treatment.

(ii) Trial 1, 1956–1957

The plants treated were first ratoon Mons Mari bananas which commenced to flower in April 1957. Treatments commenced on December 24, 1956, and continued until September 1957, by which time the bunches on the first ratoon plants had been harvested.

A randomized layout was not used. Two reasonably uniform blocks of plants were selected in a plantation near the Barron River outside Cairns. Each block consisted of 15 rows each containing 21 plants.

Three treatments were located in each block, 5 rows of 21 plants being allotted per treatment. These were as follows:

Block 1: A. Misting at weekly intervals.

B. Fogging at weekly intervals.

C. Untreated.

Block 2: D. Misting at fortnightly intervals.

E. Fogging at fortnightly intervals.

F. Untreated.

The results are summarized in Table 13.

TABLE 13

Mean Values for Number of Leaves, Percentage Leaf Spot and Bunch Weight, Trial 1, 1956–1957

Treatment	No. of Leaves		Percentage Leaf Spot		Average Bunch Weight (lb)
	Parent	Follower	Parent	Follower	
Block 1—					
Misting—weekly ..	10·2	8·7	8·4	11·1	38·9
Fogging—weekly ..	10·2	8·4	16·2	14·1	37·1
Unsprayed	8·3	6·6	18·5	15·3	29·0
Block 2—					
Misting—fortnightly ..	8·7	7·1	19·1	16·2	29·6
Fogging—fortnightly ..	8·5	6·8	21·4	15·8	31·4
Unsprayed	7·7	6·5	19·6	18·0	30·1

Unusually heavy rains in December 1956 encouraged an early build-up of leaf spot in the experimental blocks and the benefits of oil treatment were plainly visible by March 1957. The weekly treatments were of much more benefit than those applied at fortnightly intervals. This is reflected in the means shown in Table 13 for number of leaves and percentage leaf spot at flowering. The yield figures show that both misting and fogging at weekly intervals gave substantial increases in mean bunch weight. The fortnightly treatments were definitely not economical.

The control of the disease obtained on the followers was disappointing, however, and the cumulative effects noted from spray treatment of successive crops was not evident here.

Control of leaf spot was actually better than the figures show, since the oil treatments were not effective against speckle (*Mycosphaerella musae* (Speg.) Syd.) and damage from this disease contributed considerably to premature death of foliage. Also, particularly on those plants which were misted weekly, oil toxicity to the lower leaves detracted from the performance of the mixture.

(d) Phytotoxicity

In the course of the field experimental work described above notes were made on the type and severity of injury to the plant associated with the various treatments. The important features of these observations are discussed below.

(i) Water-based Mixtures

Leaf spot sprays containing white oil and malachite green have been observed to be phytotoxic under certain conditions, in particular during the wet season months when temperature and atmospheric humidity are high and overcast skies often retard the drying of the spray on the leaf surface. Three leaf symptoms are commonly seen:

(1) Tan or brown speckled patches, spots or blotches on the lower surface of the leaves. These blemishes appear to be mainly superficial and apparently are not harmful. It is thought that they are occasioned by the oil emulsion.

(2) Watersoaked areas on the older leaves which eventually become evident as large necrotic spots or dark-brown to black specks on the upper surface of the leaves. These symptoms are encountered when mixtures containing copper are used—particularly copper oxychloride. The condition was often seen when copper oxychloride alone was used for leaf spot control. This type of damage is restricted to the older foliage and becomes more evident when treatment is continued after a plant has flowered. This might be due to the accumulation of toxic residues on the tops of these leaves; again, it might be occasioned by a loss of resistance as the leaf ages, perhaps due to the gradual loss of the protective waxy coating.

(3) A bright yellowing and leaf drop of the oldest leaf or leaves. This is seen only on obviously unthrifty plants.

In addition, some fruit pitting and oil staining are likely when a coarse spray is applied to young fruit. This is not usual when plants are hand-sprayed, for care can be taken to ensure that the jet is not directed at the young fruit. It has been seen when fixed nozzles are employed, e.g. on vertical boom spray rigs. It can be corrected by using higher pressures and small nozzle orifices.

(ii) Oil-based Mixtures

Two types of symptoms have been observed on plants treated with oily mixtures, both usually on lower leaves:

(1) *Acute.*—This eventuates when a leaf receives a direct blast of the concentrate at short range. The damage quickly becomes evident as a large necrotic patch.

(2) *Chronic*.—This is a result of continued treatment with oily mixtures and is manifested by a bright chlorosis, sometimes tending towards a bronzing, of the oldest leaves. It causes premature death of this basal foliage.

The fruit are particularly prone to oil staining and marking when the misting technique is employed and great care has to be taken to ensure that the oily mist is not directed towards the bunches.

(e) Effect of the Individual Spray Constituents

The excellent control of conidial infection obtained in Jamacia by spraying every third week with either cuprous oxide or Bordeaux mixture was attributed by Leach (1946) firstly to reduction in the number of conidia produced on sprayed leaf spots and secondly to the toxic effect, on any conidia which were produced, of dew which had been in contact with spray residues. The efficacy of these two sprays in reducing conidial sporulation was demonstrated by this author, who also showed that the viability of conidia of *Mycosphaerella musicola* was much reduced after they had been suspended in dew taken from the upper surface of sprayed leaves. He concluded, therefore, that leaf spot control was "not obtained in the usually accepted manner", i.e. by the fungicidal action of the spray residue which adheres to the susceptible surfaces of young leaves.

The antisporulant effects of Bordeaux mixture were also noted by Calpouzos (1955), who, however, qualified his observations by the statement that "the length of time that the spray application exerts its inhibiting properties is closely related to prevailing weather conditions".

In North Queensland the superiority of mixtures containing white oil and malachite green over those previously tested for efficiency in leaf spot control led to an investigation of the properties of these mixtures. The spray selected for testing was copper oxychloride (1½ lb-40 gal) plus white oil (1-160) plus malachite green (1-10,000). This mixture had given results equally as good as those obtained with the other fungicides used in the final field trial. In the experiments described below the three ingredients were used singly or in pairs so that their fungitoxic and antisporulant properties could be compared with those of the complete mixture.

The white oil emulsion used in the field trials, viz. a proprietary mayonnaise type formulation containing 80 per cent. by weight refined paraffin oils, was employed in these tests.

(i) Fungitoxicity

In Experiments 1 and 2 each spray treatment was applied with a "Rega" continuous atomizer to the upper and lower surfaces of the four youngest fully opened leaves on each of three plants. A plot thus comprised one plant. There

were two control plots in Experiment 1. The leaves were sprayed once only. The observations were made within a 6 in. x 2 in. rectangle marked with a "Chinagraph" pencil in a central position on the left-hand side (i.e. the first side to unfurl) of each leaf. Before the leaves were sprayed any lesions present within these areas were marked, and at regular intervals from the date of spraying, notes were made of the numbers of fresh streaks which had appeared and of the streaks which had changed to spots.

The treatments and results are given in Table 14, where the data are expressed as mean numbers of streaks per marked rectangle for the three plants. In Figure 10 a histogram illustrates the extent and the rate of change from streak to spot associated with the various treatments in Experiment 1. The columns represent weekly counts over a period of six weeks.

TABLE 14

Toxicity Experiments 1 and 2—Total Number of Streak Lesions Appearing within 6 × 2 in. Rectangles (Expressed as Means of Results from 3 Plants)

Leaf Position (from top)	1		2		3		4	
Treatment	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Copper oxychloride (1½–40)	0	117	54	112	66	149	110	124
Malachite green (1–10,000)	95	31	87	90	83	187	89	110
Malachite green (1–5,000)	..	136	..	81	..	102	..	167
White oil emulsion (1–160)	0	27	3	134	125	133	71	81
White oil emulsion (1–160) + Malachite green (1–10,000)	0	0	26	53	159	50	120	146
Copper oxychloride (1½–40) + Malachite green (1–10,000)	16	85	41	144	46	25	63	101
Copper oxychloride (1½–40) + White oil emulsion (1–160)	0	9	38	124	34	75	91	174
Copper oxychloride (1½–40) + White oil emulsion (1–160) + Malachite green (1–10,000)	3	0	7	70	45	78	39	107
Unsprayed	161	172	140	173	136	180	89	89

The lesion counts in these experiments were very variable and the degree of control obtained, except in the case of those sprays having a suppressive action, varied with the amount of infection which had been initiated prior to the spray application. For example, the apparent anomaly in Experiment 2 where malachite green (1-10,000) gave better results than either copper oxychloride (1½-40) or malachite green (1-5,000) was quite obviously related to the length of time the first leaves had been unfolded when the treatments were applied. This was easy to determine by comparing the states of development of the unfolding heart-leaves on the various plants.

In order to reduce or eliminate this variation it was necessary to adopt an experimental design in which the treatments to be compared were randomized

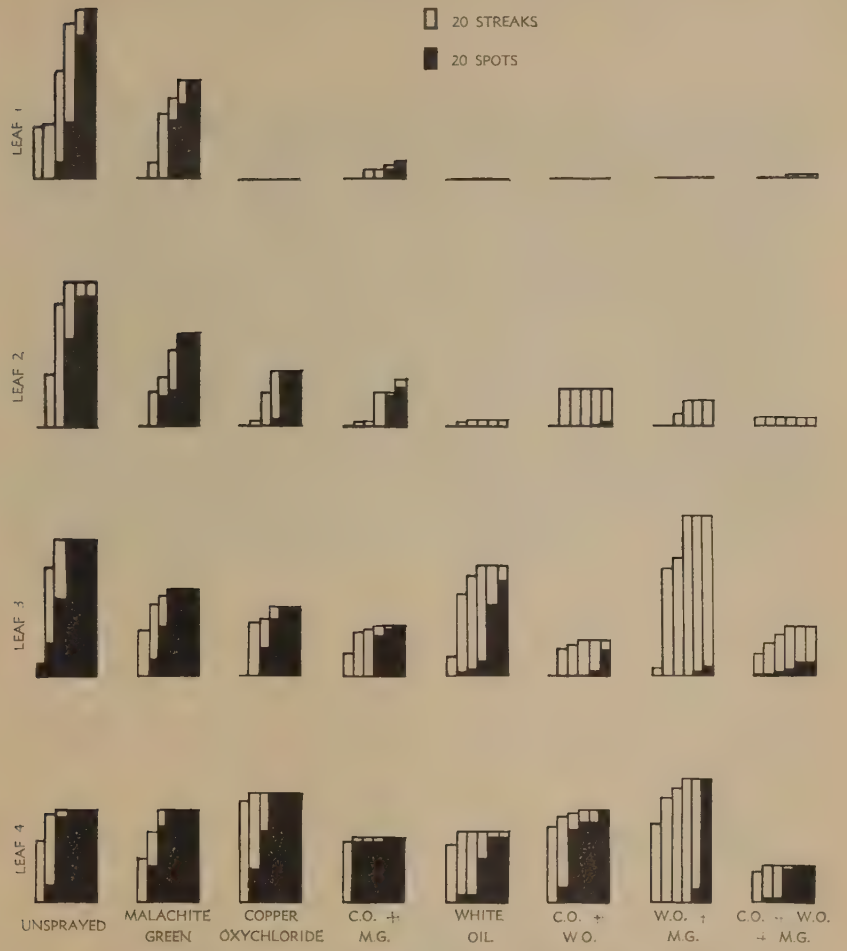

Fig. 10.—Toxicity Experiment No. 1. Mean number of streaks (white) and spots (black) per marked leaf area in weekly counts over a period of six weeks.

on a single leaf. A modification of a method used elsewhere for testing banana leaf spot fungicides (Merny 1955) was therefore used in Experiments 3, 4, 5 and 6. The fungicides were applied with a nebulizer to circles 4·5 cm in diameter drawn on the lower surfaces of the treated leaves. A single application was made when the trial commenced. Usually four treatments—three sprays and one unsprayed check—were included in each trial (Figure 11). The four circles were randomized in each of four positions on the leaf in question and this was repeated on each of four plants. Sixteen replicates of each treatment were thus provided. The four youngest fully opened leaves on each plant were treated.

In each case copper oxychloride was used at a strength of 1½ lb to 40 gal and white oil emulsion at 1-160 by volume.

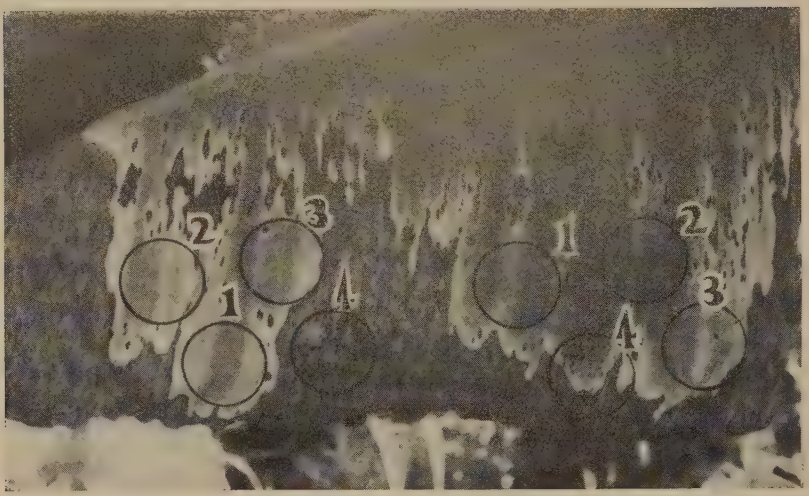

Fig. 11.—The replicated circle method, Toxicity Experiment No. 6. Control obtained by one application to first leaf. Treatments: 1, Copper oxychloride + white oil + malachite green. 2, White oil + malachite green. 3, White oil. 4, Unsprayed.

A proprietary wax emulsion at a strength of 1 in 80 was included in Experiment 3 in order to determine whether this type of paraffin compound possessed properties similar to those of the white oil emulsion.

Results were assessed by making counts at regular intervals of the numbers of streaks and spots in the treated circles. The data are detailed in Tables 15, 16, 17 and 18.

TABLE 15

Toxicity Experiment 3. Equivalent Means of Numbers of Streaks

Treatment	Leaf No.				Mean
	1	2	3	4	
1. Copper oxychloride	3·3	40·3	40·9	48·3	23·6
2. Wax emulsion (1–80)	14·7	47·5	54·6	49·0	37·2
3. Copper oxychloride + Malachite green (1–5,000)	4·7	42·0	47·5	46·8	26·5
4. Unsprayed	29·5	50·6	50·8	57·5	45·8
Means	9·4	44·9	48·2	50·3	32·1
	1, 3 << 2 2 << 4	No significant differences			

TABLE 16

Toxicity Experiment 4. Equivalent Means of Numbers of Streaks

Treatment	Leaf No.				Mean
	1	2	3	4	
1. Copper oxychloride + White oil emulsion	·3	5·4	17·6	9·4	5·3
2. White oil emulsion	2·8	5·9	11·6	21·4	8·2
3. Unsprayed	35·1	40·5	66·9	47·1	46·0
Mean	4·6	11·2	24·2	21·3	13·0
	1<<2<<3	1, 2<<3	1, 2<<3	1<<2<<3	

TABLE 17

Toxicity Experiment 5. Equivalent Means of Numbers of Streaks

Treatment	Leaf No.				Mean
	1	2	3	4	
1. Copper oxychloride + White oil emulsion + Malachite green (1–5,000)	2·1	13·9	16·5	16·3	9·9
2. White oil emulsion + Malachite green (1–5,000)	0·6	3·6	9·9	15·4	5·1
3. Malachite green (1–5,000) ..	23·9	43·1	53·1	33·1	36·7
4. Unsprayed	41·8	41·4	53·8	31·8	41·6
Mean	7·6	17·9	26·5	22·7	17·0
	2<1<<3 3<4	2<<1 1<<3, 4	1, 2<<3, 4	1, 2<<3 2<<4 1<4	

TABLE 18

Toxicity Experiment 6. Mean Numbers of Streaks

Treatment	Leaf No.		
	1	2	3
1. Copper oxychloride + White oil emulsion + Malachite green (1–10,000)	0·1	0·6	13·4
2. White oil emulsion + Malachite green (1–10,000)	0·	0·1	13·9
3. White oil emulsion	0·	0·5	16·1
4. Unsprayed	22·2	35·1	30·8

These data were not analysed, the differences being obvious for Leaves 1 and 2.

Fungicidal and/or fungistatic properties conventionally are evaluated by ability to inhibit spore germination. It was necessary to know the toxicity to leaf spot spores of each of the three constituents of the leaf spot spray formula. This was therefore investigated in a series of *in vitro* spore germination tests.

The conidia employed in these experiments were removed from mature leaf spots which had been excised from spotted leaves and incubated overnight in a saturated atmosphere at 81°F. The following technique was used to prepare the spore suspension: A drop of the liquid in which the spores were to be suspended was placed on each spot. These drops were then sucked up with a 1 ml pipette and the washings from at least six spots were transferred first to an excavated block and thence to sterile glass slides, coated with a film of plain agar (2 per cent. water agar) or with a 2 per cent. agar prepared from white oil emulsion (1-160). These slides were enclosed in petri dishes on damp cotton wool. Usually three drops were placed on each slide.

Germination counts were made after incubation at 81°F for at least 48 hr. Three hundred spores per treatment were counted.

TABLE 19

Toxicity Experiments 7, 8, 9. Effect of White Oil Emulsion, Malachite Green and Copper Oxychloride on Germination of Conidia of Mycosphaerella musicola

Spore Suspension	Germination Surface	Germination (%)
Experiment 7—		
White oil emulsion (1–160)	2% plain agar	55
Malachite green (1–10,000)	„	3
Sterile water	„	94
Sterile water	2% plain agar + white oil emulsion (1–160)	70
Experiment 8—		
Sterile water	2% plain agar	60
" Agrimycin " solution 200 p.p.m.	„	100
" Agrimycin " solution + white oil emulsion (1–160)	„	100
Experiment 9—		
" Agrimycin " solution 200 p.p.m.	2% plain agar	100
" Agrimycin " solution + Malachite green (1–10,000)	„	0
" Agrimycin " solution + White oil emulsion (1–160)	„	100
" Agrimycin " solution + Copper oxychloride ($1\frac{1}{2}$–40)	„	0
" Agrimycin " solution 200 p.p.m.	2% plain agar + white oil emulsion (1–160)	100

In preliminary spore germination tests a great variation due to bacterial contamination had been noted in percentage germination of spores removed from spots in sterile water and it had been found that this could be avoided by washing the spots lightly prior to incubation in "Agrimycin" (15 per cent. streptomycin; 1·5 per cent. oxytetracycline) 200 p.p.m. or by using "Agrimycin" solution 200 p.p.m. as the germinating medium.

The series comprised three experiments. In the third experiment the spots were washed lightly in "Agrimycin" solution prior to incubation. The treatments and results are summarized in Table 19.

(ii) Antisporulant Action

In the first series of experiments four trials were conducted. In three of these the sprays were applied to the upper surfaces of spots which had been cut from the leaves, washed in water, dried and pinned on heavy cardboard so that they could be sprayed collectively. After spraying, the spots were dried and then incubated overnight at 81°F on damp cotton wool in 4 in. petri dishes. Six spots were exposed in each dish. In the remaining trial individual leaves were treated and the required number of spots was excised as soon as the spray had dried. The spots were then incubated as above. In each of these trials 18 spots per treatment were examined.

In a second series of two trials the effect of weathering on antisporulant properties was investigated. The treatments used were those combinations which had been shown to give the best control of leaf spot. The sprays were applied to the upper surfaces of spotted leaves. One plant was sprayed with each treatment and the results were assessed by examining 36 spots at a similar stage of development from each of these sprayed plants. The conditions in these two trials were: (1) exposure for 3 days, no rain during this period, heavy dews each night; and (2) exposure for 6 days, 0·81 in. of rain on one night during the 6-day period, heavy dews on the other nights.

The method of assessment in both series was as follows: Each spot was examined with a dissecting microscope (x10 eyepiece, x4 objective) and assigned a rating for number of sporing sporodochia and intensity of sporulation on these sporodochia on the following scales:

Number		Intensity	
	Nil	0	Nil
1	1– 25%	1	Trace
2	26– 50%	2	No more than 25%
3	51– 75%	3	26– 50%
4	76–100%	4	51– 75%
		5	76–100%

These ratings were used to derive a figure for percentage sporulation for each treatment. This figure was obtained by calculating the product (mean number x mean intensity) in each trial; this was then expressed as a percentage of the maximum possible product (20) and the mean percentage sporulation for the series (in the case of the first series) or for each of the two trials (in the case of the second series) was calculated.

The results are shown in Tables 20 and 21.

TABLE 20

Antisporulant Experiments. First Series. Mean Percentage Conidial Sporulation on Sprayed Leaf Spots

Treatment	Sporulation
Copper oxychloride (1½–40)	20·4
Malachite green (1–10,000)	12·8
Malachite green (1–5,000)	0·6
White oil emulsion (1–160)	39·15
Copper oxychloride + Malachite green (1–10,000)	2·0
Copper oxychloride + White oil emulsion	22·4
White oil emulsion + Malachite green (1–10,000)	15·0
Copper oxychloride + White oil emulsion + Malachite green (1–10,000)	6·0
Unsprayed	47·0

TABLE 21

Antisporulant Experiments. Second Series. Mean Percentage Conidial Sporulation on Sprayed Leaf Spots after Exposure

Treatment	Period of Exposure	
	3 days	6 days
Copper oxychloride (1½–40) + White oil emulsion (1–160) + Malachite green (1–10,000)	6	31
White oil emulsion + Malachite green	31	58
White oil emulsion	33	60
Unsprayed	52	60

(iii) Discussion

Analyses of the data from toxicity Experiments 3, 4 and 5 were carried out in terms of the variable log (1 + x), where x is the number of streaks. In all analyses the term treatment x leaves was highly significant, indicating that the differences between treatments varied from leaf to leaf. In the tables showing mean values, differences between treatments for each leaf are shown.

The results of Experiments 1 and 2, though variable, indicated that all the fungicides tested, whether used singly or in any of the various combinations

possible, reduced leaf spot infection on the first leaf. Similarly, in Experiments 3, 4 and 5 all the sprays, including the wax emulsion, significantly reduced leaf spot infection on this leaf. The outstanding feature of these experiments, however, was the fact that sprays containing white oil emulsion alone, or in its various combinations with copper oxychloride and malachite green, significantly reduced infection on leaves which received a single application when they were respectively second, third or fourth from the top of the plant. This finding indicated that these sprays were strongly fungicidal and/or fungistatic. Evidence of a suppressive effect was provided by the observations made on rate of leaf spot development within the treated areas which are illustrated in Figures 10 and 11. White oil emulsion, white oil emulsion plus malachite green, and copper oxychloride plus white oil emulsion plus malachite green, plainly were the most potent fungistatic agents.

The results of Experiment 6 showed that there was little to choose between the complete mixture and either white oil emulsion or white oil emulsion plus malachite green for fungicidal and/or fungistatic properties. White oil plus malachite green appeared, if anything, to be slightly superior.

The effect of these mixtures in preventing or delaying the change from streak to spot stage lesions which was so pronounced in Experiments 1 and 2 was not equally evident in Experiments 3, 4, 5 and 6. This appeared to be due to the fact that in the early experiments whole leaves were treated, whereas in the later trials the sprays were applied to small circles. These small treated areas were eventually encompassed by necrotic tissue and the consequent disturbance to the physiology of the leaf apparently counteracted the suppressive action of the sprays.

The *in vitro* germination tests (Table 19) demonstrated that, contrary to expectations, white oil emulsion did not hinder germination and indicated that this additive was not fungicidal at the strength used. Moreover, it was not fungistatic in the conventional sense. Copper oxychloride (1½-40) and malachite green (1-10,000) were strongly fungitoxic.

The trials designed to investigate the extent and duration of the antisporulant effect of a leaf spot spray formula and its constituents were somewhat limited in scope. Nevertheless it was apparent that copper oxychloride (1½-40) and malachite green (1-10,000) when used alone or together caused an appreciable reduction in conidial sporulation on the upper surface of treated leaf spots which were not subjected to weathering (Tables 20 and 21). The effect of malachite green was more pronounced when the concentration was increased to 1-5,000. The effect of white oil emulsion alone was indefinite and the efficacy of mixtures containing white oil emulsion was approximately equivalent to the fungicidal ingredient or ingredients in the mixtures. A sharp reduction in the antisporulant properties of the complete mixture was associated with weathering of the spray residues on the upper surfaces of treated leaves for six days, during which period

they were exposed only to 0·81 in. of rain and to heavy dews. It is concluded, therefore, that in North Queensland plantations reduced sporulation must contribute little to the control of leaf spot obtained with this mixture. This would be particularly so during the wet season months, when spraying is often interrupted by heavy rain and it is not uncommon to have a majority of wet days in the period of four weeks between sprays.

The implications of the above work are that the efficacy of this spray mixture is due to its fungicidal and suppressive effects. The suppressive effect is dependent on the inclusion of white oil emulsion, which is plainly not fungistatic in the usual fashion.

(f) Commercial Application of Results of Control Experiments

The addition of white oil emulsion to any one of the fungicides copper oxychloride, Bordeaux mixture and zineb greatly improved its efficacy due to the suppressive action of the mixture in addition to its fungicidal properties. Malachite green was shown to be fungicidal and to have an antisporulant action, even though limited. In addition, it is a cheap ingredient. It was therefore thought preferable to retain this additive in the mixtures recommended to growers, which are as follows:

(1) Copper oxychloride (1½-40) + white oil emulsion (1-160) + malachite green (1-10,000).

(2) Zineb (1½-40) + white oil emulsion + malachite green.

(3) Bordeaux mixture (3-2-40) + white oil emulsion + malachite green.

In the control experiments the disease was checked by heart-leaf spraying at intervals of four weeks throughout the year. However, the seasonal variation in disease incidence was found to have a great bearing on the efficacy of the spray programme.

Due to the rapid build-up of infection during late summer and autumn, spraying should commence before the onset of the wet season, preferably in late November or early December, and should be continued at intervals of four weeks during the first half of the year. In the dry season, i.e. the greater part of the second half of the year, leaf spot is less damaging and unsprayed plants which flower later than July may retain sufficient foliage to enable them to fill their fruit to marketable size. It appears, therefore, that the interval between sprays can be considerably lengthened during this period.

Growers have quickly adapted their methods of plantation management to include leaf spot control measures. Most large plantations now are established on arable land and the use of tractors in leaf spot control programmes as well as for other cultural purposes has led to the adoption of a modified planting arrangement whereby the plants are spaced 5 ft apart in rows 13 ft apart.

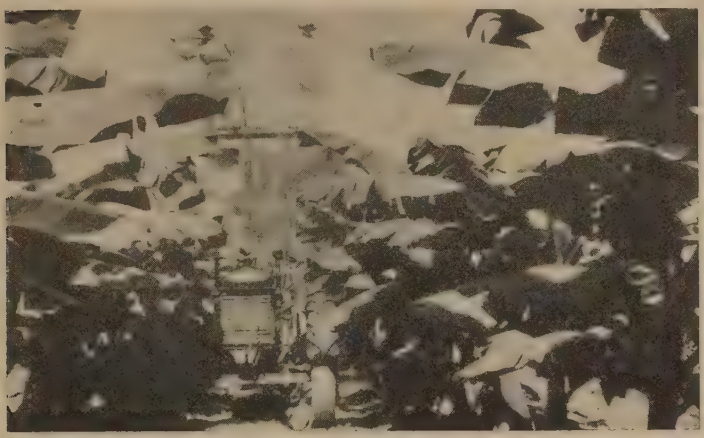

Fig. 12.—Spraying from an overhead tower on Mr. S. J. Mackay's plantation, Tully. Note complete absence of leaf spot as a result of routine operations.

The method of application on small farms is manual, using small, portable, high-pressure spray plants, pressure hosing and spray lances. Plants receive individual treatment. This method is time-consuming; 1½ ac treated per day would be a maximum. Dosages of up to 200 gal per acre may be used depending on the size of the plants.

On large plantations quicker methods of application are essential and two methods are currently in use:

(1) Overhead manual application of a spray from a tower mounted behind a tractor. The spray liquid is delivered by short lengths of pressure hosing leading to lances and nozzles held and directed by men on the tower (Figure 12).

(2) Misting row by row with air-blast misting machines towed behind tractors. This method is automatic and the spray is delivered from an arc of fixed nozzles (Figure 13).

Fig. 13.—Airblast machine operating in Mr. S. J. Mackay's plantation.

Both these methods employ reduced volumes of spray mixture. Dosages of 50-60 gal per ac are commonly used. The original formula is not altered, i.e. approximately 2 lb of fungicide and 3 pt of oil are applied per acre.

The use of air-blast machines in particular has made the treatment of banana plantations for leaf spot control most economical. The cost of materials is reduced to 18s. 6d. per acre. The estimated cost of treatment, including material, labour and depreciation, is £1 10s. per acre.

Misting or fogging with oil-based mixtures gave reasonable control of leaf spot. However, no cumulative effects were noted from continuous treatment and damage from leaf speckle disease and leaf loss due to phytotoxicity detracted from the performance of the treatments. It is considered that this method of treatment should not be adopted on plantations where water-based mixtures can conveniently be used.

No consistent advantages were gained from the use of brown paper or hessian bunch covers on sprayed or unsprayed plants. This may not apply in South Queensland, where colder conditions are experienced.

V. MODE OF ACTION OF WHITE OIL EMULSION

(a) Historical Summary

The "fungicidal" properties of mineral oils were demonstrated a long time ago (Halsted and Kelsey 1903), but until comparatively recently, and despite its widespread use as a scalicide and a sticker, oil emulsion has rarely been mentioned as a specific for plant diseases.

An oil emulsion (1 in 4 dilution) was used to control powdery mildew of rose (McWhorter 1927). Improved control of citrus black spot was obtained by the use of white oil emulsion (1-160) with Bordeaux mixture and copper oxychloride (Kiely 1950). Recently the use of one or two oil sprays at scalicidal strength in addition to copper sprays has been found to give better control of fig rust and leaf fall in South Queensland orchards (Brimblecombe 1959).

Following the discovery by Guyot and Cuillé in 1955 that straight oil misted onto bananas reduced damage by *Mycosphaerella musicola,* the role of oil in the control of this pathogen was investigated in some detail. Merny (1955) found that copper oxide and zinc coposil both suspended in oil and also oil with or without copper oxychloride in suspension gave better disease prevention than that obtained with copper oxychloride in water. All the oil treatments suppressed infection on the fourth leaf. Whether used for prevention or suppression they were most effective when applied to the lower surfaces of the leaves.

The superior effect of oil and oil/copper mixtures was later attributed by Brun (1958) to a combination of the following factors: (1) reduction in conidial sporulation; (2) reduction in spore germination on treated leaves to a point approaching zero; and (3) a check in the development of infection already established in the leaves.

There is ample evidence available in the literature to prove that fungi can remain in intimate contact with paraffin oil for considerable periods and retain their viability. Pure mineral oil is now employed as a culture preservative, this use stemming from early work which demonstrated that *Fusarium* spp. and *Alternaria* sp. remained viable under oil for six months (Sherf 1943). Since then it has been shown that many fungi can be stored by immersion in oil without fear of affecting viability (Buell and Weston 1947).

Price (1958) assessed the effects of liquid paraffin and various banana leaf spot spray oils on fungus growth by their ability to depress growth of *Fusarium oxysporum* f. *cubense* in culture. He considered the effect of spray oils on the leaf spot fungus to be partly physical and partly chemical due to the chemical nature of the unsaturated fraction. All the spray oils he tested gave a greater percentage retardation of growth than did liquid paraffin, which retarded growth by 8 per cent. compared with the control. He assumed that the effect of the same oil on a germinating *M. musicola* spore would be at least as great.

Other workers (Calpouzos, Theis, Rivera, and Colberg 1955) showed that petroleum oils which are used for leaf spot control did not reduce ascospore discharge or conidial production, nor did they inhibit germination of either conidia or ascospores; and stomatal penetration was not affected to an extent sufficient to account for the disease control obtained. These workers suggested that the oils inhibited the fungus at some stage after stomatal penetration and before the appearance of symptoms.

In a continuation of his work on the mode of action of oil in leaf spot control, Brun (1959) found that oil treatment hindered germination, germ-tube development and penetration of ascospores applied to oil-treated leaves of *Musa sinensis*. He considered it possible that the oil had a double effect, viz. (1) action on the fungus itself by which it limits its development, and (2) action on the leaf, which becomes unfit for the development of the parasite.

(b) Experimental Evidence

Some additional investigations in North Queensland on the role of white oil emulsion in leaf spot control are reported below.

(i) Effect of Time of Treatment and Leaf Surface Treated

This experiment was designed not only to provide a comparison of the relative effects of applying white oil emulsion (1-160) to the upper as opposed

to the lower leaf surface, but also to illustrate the effect on the degree of control obtained by treatment at various intervals before and after inoculation.

The emulsion was applied at the stipulated times with a nebulizer to either upper or lower surfaces in circles 4·5 cm in diameter marked on the three youngest leaves of a single plant. An aqueous suspension of conidia was atomized onto the lower surface of each circle at the required time. The results are presented in Table 22.

TABLE 22

Effect of Leaf Surface Sprayed and Time of Application of White Oil Emulsion expressed as Mean Number of Lesions per Treatment

Leaf Surface Sprayed	Weeks before Inoculation			Weeks after Inoculation		
	3	2	0	1	2	3
Lower	9	15	9	1	5	0
Upper	42	106	75	51	48	49
Nil	63	106	89	87	85	58

Clear-cut reductions in infection were obtained by oil emulsion treatments applied to the lower leaf surfaces at intervals ranging from three weeks before inoculation to three weeks after inoculation. The effect of applying the spray at similar times to the upper surface was indefinite.

This finding could have meant that the oil applied to the lower surface, i.e. the surface onto which the conidia had previously been atomized, had affected germination and penetration, whereas oil applied to the upper surface could not have had a similar effect for it was not in contact with the spores. However, oil may affect the growth of the fungus after it has entered the leaf, for oil absorption is rapid and oil soaking is evident immediately the water in the emulsion has evaporated from the leaf surface. Despite this fact, treatment three weeks prior to inoculation was as effective as treatment and inoculation on the same day. Likewise, treatment three weeks after inoculation, by which time some infection must have resulted, was as efficient as treatment and inoculation on the same day.

(ii) Effect on Spore Germination

In the course of this work it was often noted when leaf mounts were made from inoculated areas on treated and untreated leaves that spore germination and appressoria formation on the former were not appreciably different from that on the latter. One such case is shown below. The mounts were made, using the method previously described, at intervals of two and three weeks from the

date of spraying. The following were the totals for percentage germinated spores and percentage germ tubes with appressoria:

	Percentage Germination	Percentage Appressoria
White oil treated ..	76	22
Untreated	79	28

(iii) Viability of the Fungus in Treated Leaves

The fungus remains viable in lesions which have been treated with white oil emulsion. This fact was verified by many isolations from treated tissue. One such experiment is described below.

Leaves showing early (yellow) streak stage infections were treated on upper and lower surfaces with white oil emulsion applied with a "Rega" continuous atomizer. Five weeks later, during which period no further development of the streaks ensued, isolations were made from these lesions. Streaks from untreated leaves on a nearby plant were plated out also as checks.

The infected tissue was cut into strips, dipped in mercuric chloride/hydrochloric acid solution (0·5 per cent.) for 5 min, washed well in 70 per cent. alcohol and dried. The streaks were cut out, crushed with a sterile scalpel and plated. Three culture media were employed, viz. standard potato dextrose agar, 2 per cent. plain agar and acidified potato dextrose agar (2 large drops of lactic acid to 70 c.c. of medium).

The results after three weeks' incubation were:

(1) Streaks treated with oil emulsion—96 plantings, 40 *Cercospora* cultures.

(2) Untreated streaks—92 plantings, 32 *Cercospora* cultures.

(c) Discussion

The implications of the above work when considered in conjunction with that described in the section dealing with the effect of individual spray constituents are that an emulsifiable-type paraffin oil when diluted 1–160 with water and used with or without a fungicide reduced visible leaf spot infection and suppressed disease development on leaves 1, 2, 3 and 4. The emulsion did not reduce conidial sporulation appreciably, nor did it hinder germination *in vitro*. Percentage spore germination and appressoria formation on sprayed leaves were apparently not depressed. A single application of oil emulsion was sufficient to suppress growth of the leaf spot fungus up to three weeks after it had been applied and it was able to exert its effect when applied three weeks

after the spores reached the leaf. In addition, white oil emulsion, while suppressing the further development of established infections, did not affect the viability of the fungus in the treated leaf tissue.

The above evidence suggests that the suppressive action of the oil emulsion on banana leaf spot is not due to its being fungistatic in the conventional sense.

It is also of interest to note here that an emulsion of a vegetable oil, viz. cottonseed oil, has given somewhat analogous results in the control of another fungous disease, tobacco blue mould. The superior efficiency of a cuprous oxide/cottonseed oil emulsion spray for blue mould control was attributed to the action of the oil, which, in spore germination tests, was definitely not fungicidal (Clayton *et al.* 1943). These authors stated that when oil-sprayed leaves were infected "the mycelium spreads for a short time then ceases to grow."

There appear to be three possible methods of action. These are:

(1) *A purely physical effect whereby contact of the oil with the fungus inhibits the growth of the latter.* However, it has been shown in this work that spores will germinate and germ tubes grow quite readily in white oil emulsion. In addition, the leaf spot fungus grew quite readily on agar prepared with white oil emulsion as a base. Calpauzos and his collaborators (1959) also found that oils which gave complete disease control when sprayed on leaves did not inhibit growth in culture.

(2) *An inhibition by the oil of the activity of enzymes secreted by the fungus.* If there is an action of this nature then it can only function when the enzymatic activity of the fungus is at a minimum. White oil treatment does not suppress infections which have progressed to a stage of development beyond the yellow streak stage, when, presumably, enzyme secretion by the fungus is active. Also, white oil fails to prevent or delay the change of yellow streaks to spots where infected treated areas are encompassed by heavily infected untreated areas.

(3) *An effect on the physiology of the leaf tissue which results in an inhibition of fungus growth within the leaf.* White oil emulsion was noted by Kiely (1950) to delay the appearance of lesions from latent mycelia of the citrus black spot fungus. He attributed this largely to the effects of the oil in slowing down the processes of fruit maturity.

As a result of their wide use as insecticides and weedicides, some attention has been paid to the effects of oils on the physiology of plants.

Spuler, Overley, and Green (1931) commented: "Highly refined oils are not toxic to foliage in the sense that leaf tissue is destroyed, but they do under certain conditions interfere with leaf metabolism."

A number of authors have shown the oil to affect the respiration and photosynthesis of the treated plant. However, the nature of this effect varies considerably with type of oil, species of plant and method of investigation (Wedding, Riehl, and Rhoads 1952).

Most recent workers on the subject agree that photosynthesis, respiration and transpiration are inhibited after the application of oil sprays. These effects are more usually associated with oils such as citrus spray oils, which cause slow-developing chronic injury, than with the weedicide oils which cause rapid-acting, acute injury (Currier and Peoples 1954). These relatively non-toxic spray oils enter entirely or largely through stomata and usually spread widely throughout the plant (Dallyn 1953).

There are differences of opinion as to the manner in which oil effects photosynthesis. Dallyn (1953) considered that a mechanical interference by the oil with gaseous exchange or water supply is the best explanation for any interference with photosynthesis. van Overbeek and Blondeau (1954), however, suggested that the effect might be due to solvent action on the lipoid phase of the chloroplasts and that the action of oils in plants is apparently primarily a physical-chemical effect of solubilization influencing the semipermeability of the plasma membrane. Riehl and Wedding (1959) found a definite relation between inhibition of photosynthesis and increasing oil deposit. The inhibition appeared to be the result of interference with gaseous exchange caused by the presence of the spray oil. The theory of solubilization expounded by van Overbeek and Blondeau seemed to agree with experience with oil sprays on citrus in California.

Riehl *et al.* (1958) found that a single application of 1·75 per cent. oil emulsion to citrus reduced transpiration by two-thirds within 24 hr. Recovery of transpiration occurred with the dissipation of oil from the leaves, which in this case took 3–5 weeks. These authors considered that reduction in transpiration in citrus foliage after application of an oil spray was due to physical interference by the spray oil on or in the leaf tissue.

Wedding and Riehl (1958) were able to demonstrate that petroleum oil of the commercial "emulsive" spray formulation, in amounts approximating those deposited by the usual insecticidal operations, inhibits the transport of phosphate into leaves to which it has been applied. They consider the inhibiting effects of oil on translocation are probably general and non-specific and come about through physical interference with the transport mechanism either by actual obstruction of the vessels with oil or by the solubilization reaction.

It is evident, therefore, that the physiological processes could be materially affected following the application of oil emulsion to banana leaves. The disturbance to the metabolism of the leaf tissue may be responsible for the prolongation of the latency of the leaf spot fungus. The manner in which the

growth of the fungus could be affected is not known. However, the inhibiting effect of oil on photosynthesis and translocation and its influence on the semi-permeability of the plasma membrane indicates that an alteration in the carbohydrate balance of the host which possibly deprives the fungus of its favoured carbohydrate substratum could be involved. Alternatively it might mean that the action of the oil increases the resistance of the host tissue to the enzymes or toxins secreted by the fungus.

The fact that oil has little or no effect on lesions which have developed beyond yellow streaks to the stage where the fungus is vigorously parasitic indicates that the activities of the fungus have disorganised the functioning of the parasitised leaf tissue to the extent where photosynthesis and other normal physiologic processes have ceased.

In infected treated areas encompassed by heavily infected untreated areas, the failure of white oil to prevent or delay the change of yellow streaks to spots while preventing the development of streaks is of interest but without adequate explanation.

Some preliminary work attempting to control the physiological aging of leaf tissue by growth regulators has been carried out but no positive results have yet been obtained.

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THREE LEAF SPECKLE DISEASES OF THE BANANA IN QUEENSLAND

By W. PONT, B.Sc.*

SUMMARY

Leaf speckling diseases of the banana caused by *Chloridium musae* Stahel, *Ramichloridium musae* Stahel and *Mycosphaerella musae* (Speg.) Syd. are described.

The first two diseases appear to be confined to the tropical north of Queensland where they are present as relatively superficial leaf blemishes in rain-forest plantations or on native bananas. Mycosphaerella speckle, however, causes serious damage in plantations in Queensland and New South Wales.

A conidial stage of *M. musae* has not yet been found. The disease is disseminated by ascospores ejected from perithecia in the dead leaf tissue. Germination takes place on the lower surface of the leaves. After a lengthy period of epiphyllic existence, at least 5-6 weeks, appressoria are formed and the fungus penetrates the leaf through the stomata on the under-sides.

In experiments with protectant fungicides, Mycosphaerella leaf speckle was controlled by a single application of either copper oxychloride or zineb to the lower surfaces of leaves not showing visible symptoms. Other sprays which controlled Mycosphaerella speckle when applied to the under-sides of the leaves were white oil emulsion, white oil emulsion/malachite green, phenyl mercuric chloride and a straight paraffin oil. Treatments applied to the upper surfaces of the leaves were ineffective.

Tests with eradicant sprays showed that sodium pentachlorphenate almost completely inhibited ascospore discharge when sprayed onto the lower surfaces of dead leaves containing mature perithecia.

I. INTRODUCTION

Leaf speckling diseases have been described on several banana varieties in the West Indies and Australia. In Queensland a leaf speckle was first described on the Cavendish variety by Simmonds (1933), who found a species of *Mycosphaerella* to be consistently associated with the disease. In Trinidad the fungi *Nigrospora oryzae, Gloeosporium musarum, Stachylidium* sp., *Fusarium* sp. and *Penicillium* sp. were isolated from a characteristic leaf mottling or speckling of leaves of Cavendish, Gros Michel and Lacatan bananas by Wardlaw (1935), who suggested that this might be the same disease as described by Simmonds.

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Two distinct leaf speckle diseases of the Congo banana caused by the closely related fungi *Chloridium musae* Stahel and *Ramichloridium musae* Stahel were later described from Surinam (Stahel 1937). Stahel suggested that Simmonds' Queensland speckle might be similar to that caused by *Ramichloridium musae* in Surinam. *Chloridium musae* was subsequently recorded in Fiji (Parham 1938) and Brazil (Bitancourt 1940). The list of speckling diseases was further extended when Martyn (1945) described a "flecking" caused by *Cladosporium musae* Mason. Martyn also confirmed the presence of Stahel's Chloridium speckle in Jamaica and described a peculiar epiphyllic fungus commonly found on banana leaves in Jamaica and which was named *Zygophiala jamaicensis* by E. W. Mason of the then Imperial Mycological Institute.

In Queensland the leaf speckle originally recorded by Simmonds (1933) and referred to in this article as Mycosphaerella speckle is a common cause of defoliation. Simmonds contended that in South Queensland it sometimes was "equally if not more responsible for the leaf damage occurring" than leaf spot (*Mycosphaerella musicola* Leach). In North Queensland, where leaf spot outbreaks are more virulent and more sustained, the importance of Mycosphaerella speckle has, until recently, been overshadowed by that of leaf spot. Improved control measures for leaf spot now in use in this region of the State have been responsible for a marked reduction in the amount of damage due to this cause. However, in many cases and for reasons which will be discussed later, the incidence of leaf speckle has not been similarly depressed by these measures and the damage attributable to this disease has become more obvious. The increased importance of Mycosphaerella speckle necessitated further investigations into its cause and control and these questions are the main subjects dealt with in this paper.

In the course of this work it became apparent that two other leaf speckle diseases often occur in North Queensland banana plantations, viz. those originally described by Stahel in Surinam and caused by *Chloridium musae* and *Ramichloridium musae* respectively. These two disorders are referred to in this article as tropical leaf speckles. This is the first Australian record of *C. musae* and appears to be the only published record of *R. musae* since it was described by Stahel.

It is of interest to note here that the epiphyllic fungus *Zygophiala jamaicensis* Mason has also been recorded on leaves of Cavendish and Mons Mari bananas in North Queensland.

II. TROPICAL LEAF SPECKLES

(a) Chloridium musae and Ramichloridium musae

Both these diseases are commonly found in plantations in the high-rainfall area which extends from Tully to Deeral in North Queensland. Such plantations are located on clearings in rain-forest and the protection afforded by the trees favours speckle development presumably because of the limited air movement and

high humidity. The two diseases have been found on the Mons Mari, a Cavendish mutant commonly grown in this area, and also on a native species, *Musa banksii* F. Muell. Both fungi are often seen in mixed culture on the one leaf.

(b) Symptoms and Effect on the Host

(i) Chloridium musae

This fungus produces on green leaves chlorotic blotches roughly circular in outline and up to 1½ in. in diameter. On the upper surface of these blotches dark-brown or black pin-point sized specks are clearly visible and are thickly distributed (Figure 1). On the lower surface of the lesions the specks are not nearly as prominent and when an affected leaf is viewed from underneath the disease appears as tan-coloured blotches. If a piece of speckled leaf tissue is bent around the finger with the lower surface of the leaf uppermost and held against the light it is easy to see, even with the naked eye, the densely packed, bristle-like sporophores of the causal fungus.

Fig. 1.—Upper surface of leaf of Mons Mari variety showing lesions of *Chloridium musae*.

(ii) Ramichloridium musae

On green leaves the lesions are quite obvious as irregularly circular, dark-grey to black patches on the lower surfaces (Figure 2). These patches can be resolved with a hand lens into densely aggregated, minute black specks. The individual speckled areas are smaller than those produced by *C. musae* but often they merge into quite extensive smoky blotches. The blotches are still discernible but less distinct on the upper surfaces of the leaves and here a speckled pattern cannot be distinguished. The sporophores are clearly visible on the lower surface of the lesions as a dense, almost velvety coating.

These fungi are weak parasites and are seen only in unsprayed plantations. Their effect on the host is hard to assess because damage from either leaf spot or Mycosphaerella speckle is always present on the older leaves and confuses the

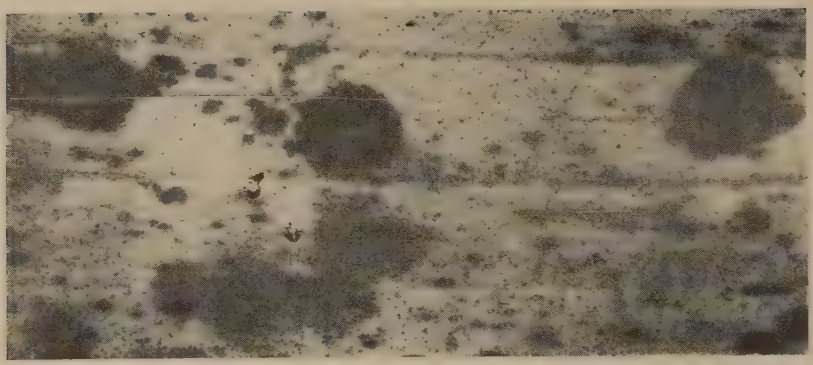

Fig. 2.—Lower surface of leaf of Mons Mari variety showing lesions of *Ramichloridium musae* (above) and *Chloridium musae* (below).

issue. Lesions of these tropical speckles are often seen on young foliage, even as high on the plant as the third fully expanded leaf, where leaf spot or Mycosphaerella speckle symptoms are not yet evident. However, no extensive necrosis or breakdown of leaf tissue which could lead to premature defoliation has ever been associated with infection by either organism.

(c) Cultural Characteristics

Both fungi can be easily isolated by removing fruiting structures from a lesion with a sterile needle. Both can be cultured on potato dextrose agar. Colonies of *R. musae* on this medium grow slowly; they are dark-grey with an elevated centre on which, as the cultures age, a lighter grey aerial mycelium is produced. *C. musae* grows faster and forms flat or only slightly elevated colonies which are wrinkled and of a similar dark-grey colour (Figure 3). Both fungi produce sporophores and spores on potato dextrose agar; the sporophores of *R. musae* are, however, usually only sparingly branched in culture.

(d) Growth-Temperature Relationships

The temperature relationships of these fungi were determined by exposing potato dextrose agar cultures in a multi-temperature incubator giving 14 temperature regimens ranging from 10°C to 37°C. Uniform squares of inoculum were placed in a central position on this medium in 4 oz clear bottles and incubated at room temperature for three days. The cultures were then examined and their outlines were marked. Two bottles were placed in each of the 13 chambers of the multi-temperature incubator. The growth was measured after 7 and 14 days' exposure (Figure 3). The results are shown in Figure 4, where the average increase in colony diameter expressed in millimetres is plotted against temperature.

Fig. 3.—Effect of temperature on growth in culture of leaf speckle fungi. Above, *Ramichloridium musae*. Middle, *Chloridium musae*. Below, *Mycosphaerella musae*.

The optimum temperature for the development of *R. musae*, 27°–28°C (82 F), was slightly higher than that for *C. musae*, 26°C (79°F), which in this trial had the same optimum as *Mycosphaerella musae*. Growth of both species was negligible below 10°C and above 35°C.

The curves for the three species *R. musae*, *C. musae* and *M. musae* were very similar in shape, and difference in temperature requirements for growth therefore appears an unlikely reason for the restricted distribution of the two tropical species.

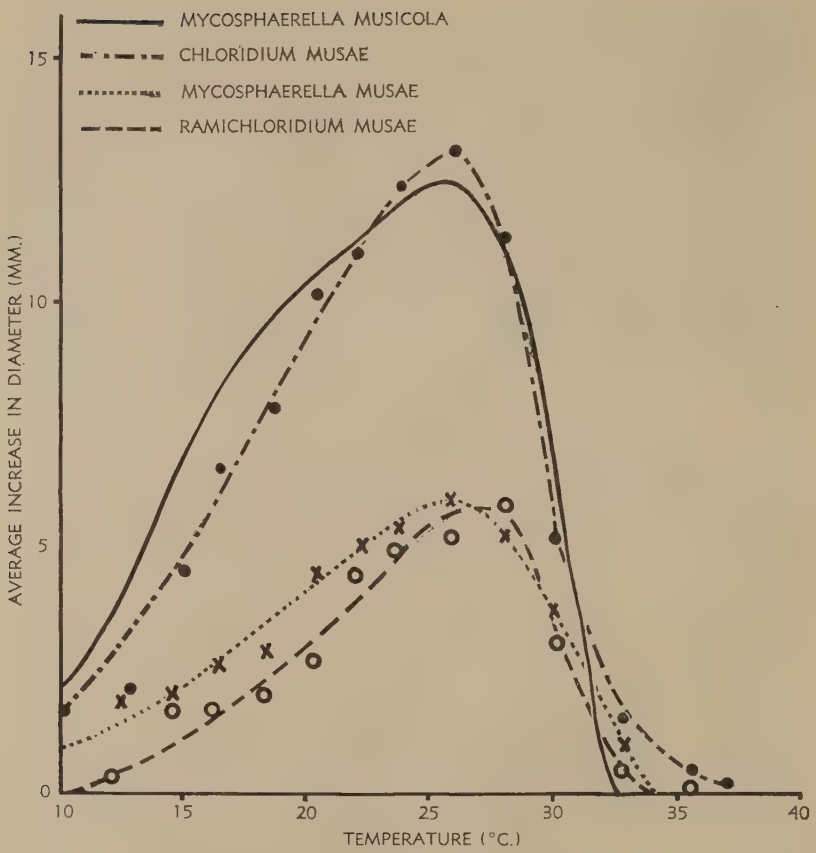

Fig. 4.—Growth-temperature curves. Each 7 days' growth on potato dextrose agar except *Mycosphaerella musicola,* which is 24 days' growth. (*M. musicola* values after Simmonds 1933).

(e) Morphology

The dimensions of the fruiting structures of the two fungi present in North Queensland are compared with those given by Stahel for his Surinam collections in Table 1.

The spores of *C. musae* are oval and those of *R. musae* are elliptical. The sporophores of the latter are longer than those of the former. The conidia of *C musae* have the papilla described by Stahel at the point of attachment to the sporophore.

TABLE 1

Dimensions of Fruiting Structures of C. musae and R. musae, North Queensland and Surinam Collections

Organism	Origin	Spores (μ)	Sporophores (μ)
Chloridium musae	Surinam	6–7·5 x 2·5–3	100–300
	North Queensland ..	6–7 x 3–4	118–291
		Average (50) 6·4 x 3·1	
Ramichloridium musae	Surinam	6–7·5 x 1·5–2·5	200–500
	North Queensland ..	6·4–9·6 x 2–3	342–411
		Average (50) 7·2 x 2	

III. MYCOSPHAERELLA SPECKLE

(a) Distribution

The only definite descriptions of this disease are from Australia, where it is present in northern and southern Queensland and in New South Wales. Material typical of the leaf speckle present in New South Wales and collected at Tullera, near Lismore, was recently examined. It was indistinguishable in appearance from the disease in North Queensland.

It is uncertain whether the leaf speckling of the West Indies referred to by Wardlaw (1935) is identical with the Queensland disease, as he made no record of a Mycosphaerella amongst the various associated organisms. The causal organism of the Queensland speckle had, however, previously been described from the Dominican Republic in the same general region (Ciferri and Fragoso 1927).

Simmonds collected material closely resembling the Queensland speckle at Peradeniya, Ceylon, in 1932 (Simmonds 1933). Recent examination has shown a Mycosphaerella closely resembling *M. musae* to be associated with this material (Table 3), and it would appear that the two diseases are identical. In spite of the paucity of references to its occurrence, Mycosphaerella speckle is therefore widely distributed throughout the banana-growing regions of the world.

(b) Host Range

All the varieties commonly grown on a commercial scale in Queensland, viz. the Cavendish and its two mutants Mons Mari and Williams Hybrid, also the Lady Finger and Sugar, are susceptible. The variety called Ducasses in Queensland, which is immune to leaf spot (*Mycosphaerella musicola*), is susceptible. The disease is also commonly recorded on a native banana (*Musa banksii* F. Muell).

(c) Symptoms

The first signs of infection on a leaf are clearly visible early in the morning when dew is present or during rainy periods as watersoaked patches which exude droplets of moisture. In the absence of moisture the infected areas can often first be distinguished on the lower leaf surface as light-brown or tan-coloured irregular blotches which may show on the upper surface as smoky patches. As the disease progresses the blotches on the lower surface darken in colour, become visibly stippled due to necrosis of the stomata and cells of the spongy parenchyma, and eventually become irregularly shaped speckled areas visible on both leaf surfaces (Figures 5 and 10). The colour of these speckled patches ranges from dark purple to black. By this time the leaf tissue in and

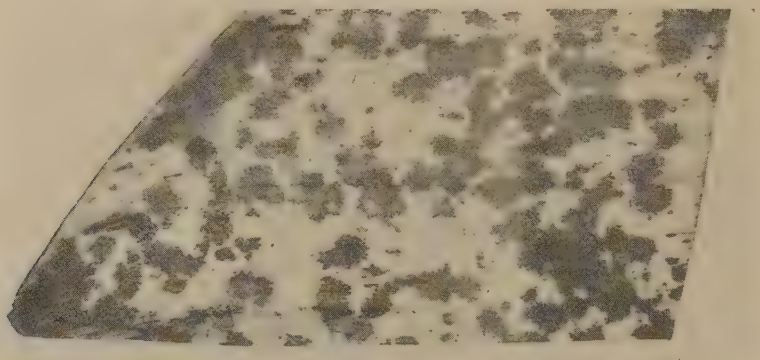

Fig. 5.—*Mycosphaerella musae.* Natural infection on lower surface of leaf of *Musa banksii.*

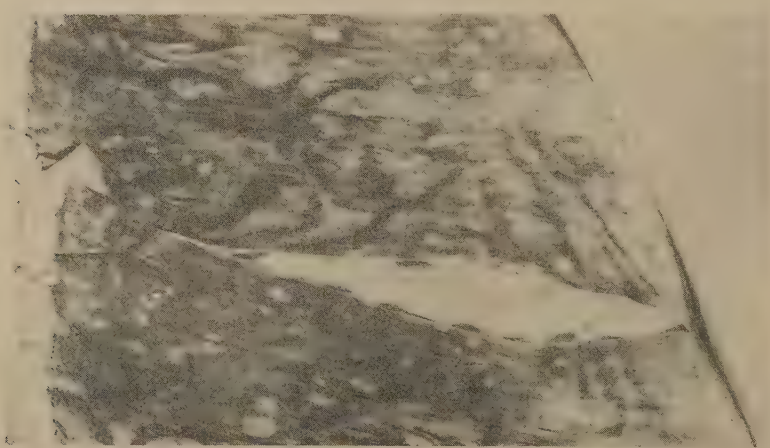

Fig. 6.—*Mycosphaerella musae.* Upper surface of dead leaf of Ducasses variety.

around the speckled area is chlorotic. As the disease progresses the individual specks of necrotic tissue coalesce to give large necrotic blotches which bleach with age as the affected area dries out and become grey on the lower surface and straw-coloured on the upper surface. Usually at this stage the perithecia first become visible on the lower leaf surface.

Sometimes on the variety Ducasses local patches of speckle may give rise to small necrotic spots with a dark-brown or black margin, light-tan on top and grey below (Figure 6). Perithecia are numerous on the lower surfaces of such spots but sparse on the upper surfaces.

On dead leaves the speckled areas stand out, being visible on the undersides of the leaves as grey blotches studded with minute perithecia (Figure 7). These structures are much less numerous on the upper surfaces of the blotches, which are usually straw-coloured.

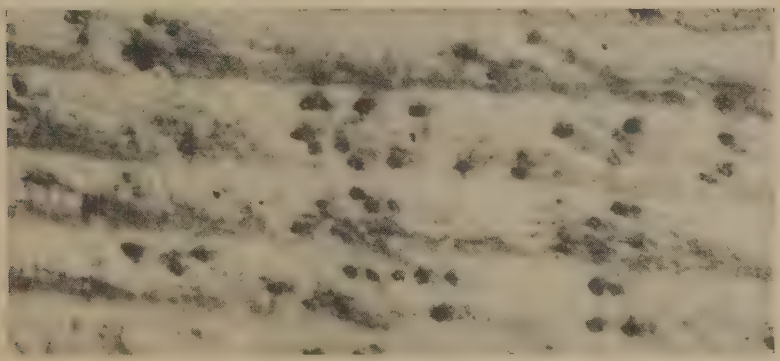

Fig. 7.—Lower surface of leaf of Ducasses banana showing perithecia of *M. musae* (X52).

Except under unusual circumstances, such as on unthrifty plants during humid, rainy weather, visible speckle lesions seldom appear above the fifth or sixth fully opened leaf and extensive necrosis and death of leaf tissue are not often seen above the ninth leaf. However, after a plant has flowered the leaves are killed off successively from the oldest upwards and quite extensive defoliation can result before the bunch is mature.

(d) Etiology

In Queensland the presence of a fungus in speckle-affected tissue was noted by Simmonds (1933). He was able to isolate consistently an organism which produced on potato dextrose agar a mound-like and slowly spreading colony with a grey compact mycelium which may later develop pinkish areas. In addition, he recorded the association of small perithecia of the Mycosphaerella type with "speckle infected areas on leaves which have been dead and dry for some time."

In New South Wales (Anon. 1945) the view was originally held that speckle was a secondary development of the injury caused by red spider (*Tetranychus* sp.). More recently observations in that State have shown that serious infection occurred in a relatively dry season in the apparent absence of red spider (Anon. 1958).

(e) Isolation of the Causal Organism

The variety Ducasses was used as a source of ascospores in order to avoid confusion with *Mycosphaerella musicola.* Spores were obtained by placing pieces of dead leaf tissue containing perithecial material into the lids of 4 in. petri dishes over a shallow layer of moist cotton wool so that when the lids were fitted the pieces of leaf were exposed above either slides coated with plain agar (2 per cent. water agar) or a film of plain agar in the bottom of the dishes. A single-spore isolator (Leach 1955) was used to remove the ascospores.

The material used for tissue isolations was always taken from definite speckled patches on living leaves. Such material was surface sterilized by dipping in a 0·5 per cent. aqueous solution of mercuric chloride/hydrochloric acid (20 g–100 c.c.) for 5 min, then washed with 70 per cent. alcohol and dried. Small pieces of the leaf tissue were crushed and transferred to plain agar in petri dishes. The resulting colonies were sub-cultured to potato dextrose agar. All isolates were cultured on this medium for purposes of comparison.

The "mound-like" fungus colony described by Simmonds was consistently isolated from speckle lesions on the Ducasses, Cavendish and Mons Mari varieties. In addition, a similar fungus was obtained when ascospores ejected from perithecia on dead speckled leaves of Ducasses and Lady Finger plants were used as inoculum. No spores are produced in culture.

(f) Pathogenicity Tests

Inoculations to prove pathogenicity were made in several ways, using vegetative and ascospore material.

(1) *Vegetative Material.*—Small pieces of agar and mycelium were transferred from cultures on potato dextrose agar to cuts made with a sharp scalpel on the lower surface of a leaf along the junction of lamina and midrib. The cuts paralleled the midrib and were essentially slits into which a wad of inoculum was placed.

(2) *Ascospore Material.*—Leaf discs were cut from infected dead leaves of the Ducasses variety showing abundant development of perithecia. These leaf discs were fitted into the lids of 4 in. petri dishes over damp cotton wool as before. The lids were inverted and clamped tightly onto the surface of a leaf with the aid of small G cramps (2 in.). A 4 in. square of $\frac{3}{16}$ in. plywood

pressed against the opposite leaf surface provided a base on to which the lid could be clamped (Figure 8). If the lower leaf surface was inoculated the lamina was inverted so that this surface was uppermost. To provide a check on the identity of spores which landed on the leaf beneath the leaf disc, $\frac{7}{8}$ in. glass coverslips were stuck to the leaf with a small drop of mucilage. When the leaf was uncovered, the coverslip was removed, flooded with cotton blue in lactophenol, inverted and placed on a slide for examination.

Fig. 8.—Inoculation technique used with *Mycosphaerella musae.*

In an alternative method, ascospores were collected by exposing suitable leaf discs over a very small quantity of water in the bottom of a petri dish. The spore suspensions from a large number of such dishes were combined and atomized onto the inoculation sites with a nebulizer. The suspension was allowed to dry on the leaf, then the inoculation site was moistened by atomizing with sterile water and a petri dish lid was tightly clamped as described above over the inoculated area.

Early experiments were carried out on plants in the open and some confusion was caused by natural infection. To reduce to a minimum the chances of infection from airborne spores, inoculations were carried out subsequently on two Cavendish plants which were growing in a lath-house situated in a sheltered position. The results of some of these pathogenicity tests are summarized in Table 2 and illustrated in Figures 9 and 10.

TABLE 2

Results of Pathogenicity Tests with *Mycosphaerella musae*

Date of Inoculation	Type of Inoculum	No. of Inoculations and Site	No. of Positive Infections	Incubation Period (days)
17–xi–1956 ..	Vegetative. Culture 1101 ex leaf lesion on Ducasses	8 wounds along midrib of youngest expanded leaf (lower surface)	8	102
17–xi–1956 ..	Vegetative. Culture 1112 from ascospores ex Ducasses	8 wounds along midrib of youngest expanded leaf (lower surface) (Fig. 9)	8	102
17–xi–1956 ..	Ascospores collected from Ducasses	Atomized onto four 4 in. dia. circles on youngest expanded leaf (lower surface) (Fig. 10)	1	102
13–xii–1956 ..	Ascospores collected from Ducasses	Atomized onto three 4 in. dia. circles on youngest expanded leaf (lower surface)	2	80
12–iv–1957 ..	Vegetative. Culture 1101 ex leaf lesion on Ducasses	12 wounds along midrib of 8th expanded leaf (lower surface)	5	86
12–iv–1957 ..	Vegetative. Culture 1104 from ascospores ex Ducasses	12 wounds along midrib of 8th expanded leaf (lower surface)	1	86
12–iv–1957 ..	Vegetative. Culture 1234 Re-isolation from positive inoculations made with ascospores 17–xi–1956	12 wounds along midrib of 8th expanded leaf (lower surface)	6	86
21–i–1958 ..	Ascospores from Ducasses	Leaf discs exposed on two sites on each of :—		
		1st expanded leaf, lower	1	83
		2nd expanded leaf, lower	2	
		6th expanded leaf, lower	1	
29–i–1960 ..	Ascospores from Ducasses	Leaf discs exposed on :—		
		1st expanded leaf, lower*	1	98
		1st expanded leaf, upper		
		2nd expanded leaf, lower		
		2nd expanded leaf, upper		
		3rd expanded leaf, lower*	1	
		3rd expanded leaf, upper		
		4th expanded leaf, lower		
		4th expanded leaf, upper		

TABLE 2—*continued*

Results of Pathogenicity Tests with *Mycosphaerella musae*

Date of Inoculation	Type of Inoculation	No. of Inoculations and Site	No. of Positive Infections	Incubation Period (days)
22–iii–1960 ..	Ascospores from Ducasses	Leaf discs exposed on :—		
		1st expanded leaf, lower†	Blotch stage only	45
		2nd expanded leaf, lower†	1	45
		3rd expanded leaf, lower		
		4th expanded leaf, upper†		
		4th expanded leaf, lower†	1	45
		5th expanded leaf, upper		
		5th expanded leaf, lower		

*The inoculation sites marked thus were injured by pricking the lower leaf surface with a battery of pins three weeks after inoculation.

†The inoculation sites marked thus were exposed to a saturated atmosphere each night for the duration of the experiment by covering with a petri-dish lid containing damp blotting paper.

Many of the inoculations made in the course of this work were inconclusive and are not recorded here. Vegetative material planted in wounds very often gave rise to a watersoaked lesion on and around the wound site. Ascospore inoculations similarly in many cases often failed to reproduce typical speckle lesions. Despite this fact, when inoculated leaf surfaces were examined large numbers of germinated spores and a profuse development of epiphyllic mycelia and appressoria could be seen. Often such artificial infections reached the stage where a light-tan blotch became visible on the inoculation site and no further development ensued.

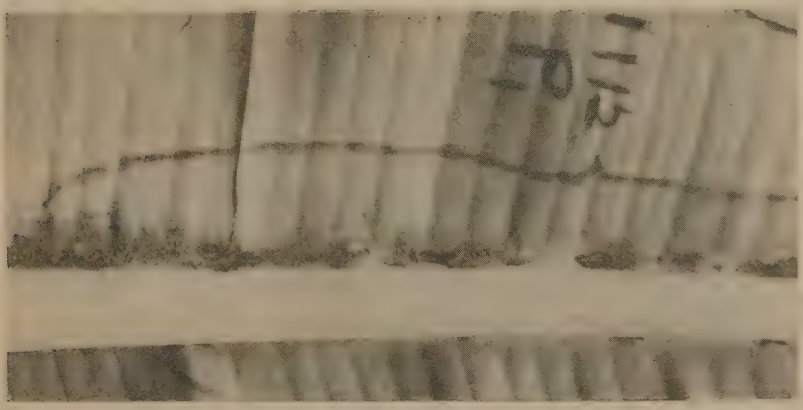

Fig. 9.—*Mycosphaerella musae*. Result of inoculating mycelium into wounds along the midrib of the youngest expanded leaf. Inoculated 17.xi.1956. Photographed 14.iii.1957.

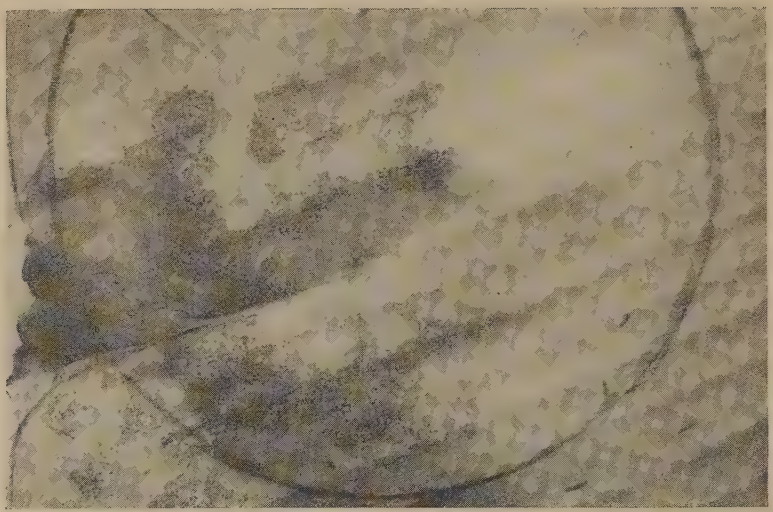

Fig. 10.—*Mycosphaerella musae*. Result of inoculating ascospores onto the lower surface of the youngest expanded leaf. Inoculated 17.xi.1956. Photographed 14.iii.1957.

The typical light-grey fungus producing a mound-shaped colony was usually re-isolated from the lesions produced. However, in the case of re-isolations from inoculations into wounds it was difficult to obtain a culture free from bacterial contamination.

Proof of pathogenicity is considered to be provided by the tests of November 17, 1956, and April 12, 1957, in which it was demonstrated that ascospores collected from speckle lesions on dead leaves of a Ducasses plant produced typical symptoms of the disease on a Cavendish plant. The fungus which was isolated from the lesions on the latter plant in turn reproduced the usual symptoms when it was inoculated into leaf tissue of a Cavendish banana through wounds. In the course of these pathogenicity tests the disease was never reproduced by the inoculation of the upper surface of a leaf with ascospores.

(g) Cultural Characteristics and Morphology

All fungus mounts were made in cotton blue/lactophenol. In order to examine the development of the fungus on the leaf surface two types of preparation were employed. In the first method the leaf portion was placed on a sheet of plate glass with the surface to be examined—usually the lower leaf surface—facing downwards. As much of the mesophyll and vascular strands as could be removed without rupturing the lower epidermis was then taken off by abrading with the blunt side of a scalpel blade. Sample pieces of the tissue remaining, cut to fit under a $\frac{7}{8}$ in. coverslip, were then cleared by warming

in lactophenol and stained in cotton blue. In the second method whole leaf pieces were decolourized by boiling in a solution consisting of equal parts of absolute alcohol and glacial acetic acid or of 5 per cent. lactic acid in absolute alcohol. The leaf pieces were then cleared by heating in lactophenol in a water-bath and stained in cotton blue before mounting in lactophenol; or alternatively, they were transferred from the decolourizing solution to cotton blue in lactophenol and heated for a few minutes before they were mounted in lactophenol.

Transverse sections made to investigate the development of the fungus within the leaf and to show details of the fruiting structures of the causal fungus were cut by hand with a blade razor and stained in cotton blue before mounting in lactophenol. In these preparations mycelia and other fungus structures stained a darker blue than did the leaf tissue.

A colony of the speckle fungus on a potato dextrose agar slope first assumes an irregularly circular outline and a smoothly convex elevation, giving it a characteristic domed shape. The colony consists of a thin layer of compact, light-grey, aerial mycelium on a hard, black, sclerotium-like hump, and its colour is grey when viewed from above and black when seen from below (Figure 3). The aerial mycelium consists of densely aggregated, sparingly branched, septate hyphae not exceeding 3μ in diameter.

As the culture ages its outline becomes more irregular and tends to elongate along the length of the tube. The edges are crenate in places. Droplets of dark-brown or black fluid may exude from any part of its elevated surface. After 6–8 weeks a pink colour develops in the grey surface mycelium. The colony on potato dextrose agar is very similar to that of the related banana leaf spot organism, *Mycosphaerella musicola.*

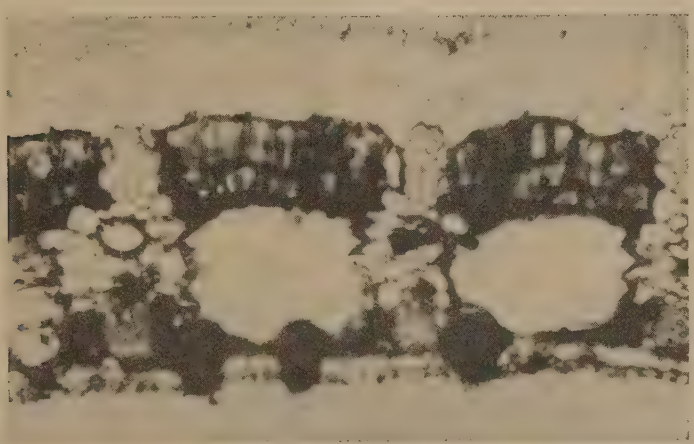

Fig. 11.—Freehand section of banana leaf with perithecia of *M. musae* on lower surface (X120).

No fruiting structures have been noted in culture. On the plant there is no apparent conidial stage and the fungus reproduces by means of ascospores. The absence of a conidial stage is not unusual, for it has been pointed out that some species of *Mycosphaerella* do not possess conidial stages (Wolf and Wolf 1947).

Fig. 12.—Freehand section of banana leaf including a perithecium of *M. musae* (X285).

Fig. 13.—Squashed perithecium of *M. musae* showing asci (X285).

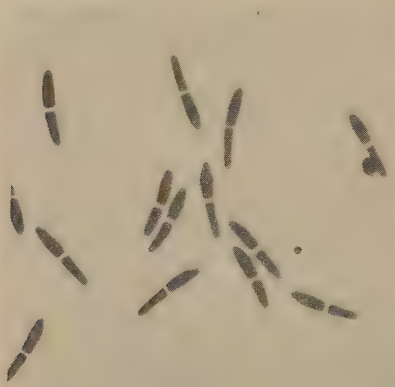

Fig. 14.—Ascospores of *M. musae* (X570).

Fig. 15.—Ascospores of *M. musicola* for comparison (X570).

The perithecia are minute (34–99μ dia.), thickly scattered, immersed, sub-globose, ostiolate, black, thin-walled and aparaphysate (Figures 11, 12). The asci are obclavate, measure 24–44μ x 8–12μ and contain 8 ascospores (Figure 13). The ascospores are hyaline, obtuse fusoid or cylindrical, 2-celled with one cell commonly broader than the other; the septum is very prominent and there is no constriction. They measure 8·8–16μ x 2·0–3μ (Table 3 and Figures 14, 15).

TABLE 3

Dimensions of Fruiting Structures of Various Species of *Mycosphaerella* on Banana

Fungus and Authority	Perithecia (μ)	Asci (μ)	Ascospores (μ)
1. *Spnaerella musae* Spegazzini Spegazzini, C. 1909. Ann. Mus. Nac. Buenos Aires XIX, p. 354	80–90	38–40 x 6–8	12–13 x 2
Ciferri, R. and Fragoso, R. G. 1927. Bol. R. Soc. Esp. Hist. Nat. 27 : 165–77			13 x 3
2. *Sphaerella musae* Saccardo Saccardo, P.A. 1917. Notae Myc. XXIII Accad. Veneto-trent, p. 67. (from Sylloge Fungorum 24 : 879)	150–180	45–50 x 7·5–9	10–12 x 2·3–2· 5
3. *Mycosphaerella Musae* (Speg.) Syd. Sydow, H. and P. 1914. Philipp. J. Sci. 8 : 482 ..		35–48 x 12–16	12–15 x 3–4
4. *Mycosphaerella musicola* Leach Leach, R. 1941. Trop. Agriculture, Trin. 18 : 91–5	46·8–72 Av. 61·8	28·8–36 x 8–10·8	14·4–18 x 3–4 Av. 16·7
W. Pont, North Queensland material (Fig. 15)			14·4–19·2 x 3·2–4·8 Av. (50) 16·3 x 3·8
5. *Mycosphaerella minima* Stahel Stahel, G. 1937. Trop. Agriculture, Trin. 14 : 257–64	25–37	24–28 x 10–12	20–22 x 5–6
6. *Mycosphaerella* sp. from Queensland speckle.			
J. H. Simmonds (3 collections)	34–81 x 45–99 Av. (84) 56 x 69·8	24–44 x 8–12 Av. (41) 30·1 x 9·1	10–15 x 2–3 Av. (63) 12·2 x 2·6
W. Pont (2 collections) (Fig. 14)	60–90		8·8–16 x 2–3 Av. (75) 13·2 x 2·7
7. *Mycosphaerella* sp. from Ceylon speckle. Collected by J. H. Simmonds, 1932.			
W. Pont	41–80	28·8 x 9·6	9·6–12·8 x 2·5–3

The spores germinate on the leaf surface. The germ-tubes grow out from both ends of the ascospore, often simultaneously. They are fine structures not exceeding 1·5μ in width. They grow quickly and branch often, giving rise to extensive, equally fine, much-branched epiphyllic mycelia. After a prolonged period of epiphyllic existence, generally of 5–6 weeks' duration, but sometimes shorter or considerably longer, appressoria are formed and the fungus penetrates into the leaf by way of the stomata (Figures 16, 17). The appressoria are stout, brown, lobed or digitate structures which are formed around or on

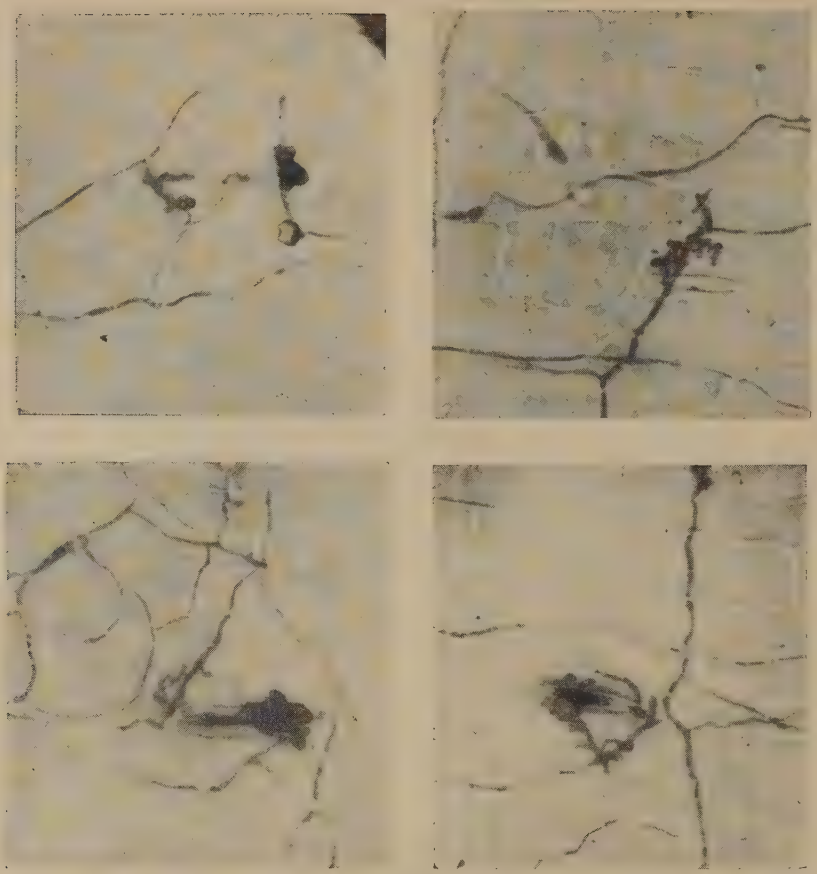

Fig. 16.—Artificial infection with *M. musae*. Germinated ascospores, epiphyllic mycelium and appressoria. Thirty-eight days after inoculating the fifth leaf (X384).

the stomatal pores. At this stage necrosis of the stomatal guard cells commences and they assume a light-brown colour. Stout brown infection tubes 3μ or more in diameter arise beneath the appressoria and penetrate through the pores into the substomatal chambers (Figure 18). The terminal cells of the infection tubes are often rounded or campanulate. From the bases of these infection tubes coarse, hyaline, intercellular hyphae up to 3μ thick grow through the mesophyll and across or along the walls of the air chambers to the palisade tissue. These intercellular hyphae branch as they grow and develop finger-like processes, presumably absorptive in function. The lateral hyphae are finer and may not exceed 1μ in thickness.

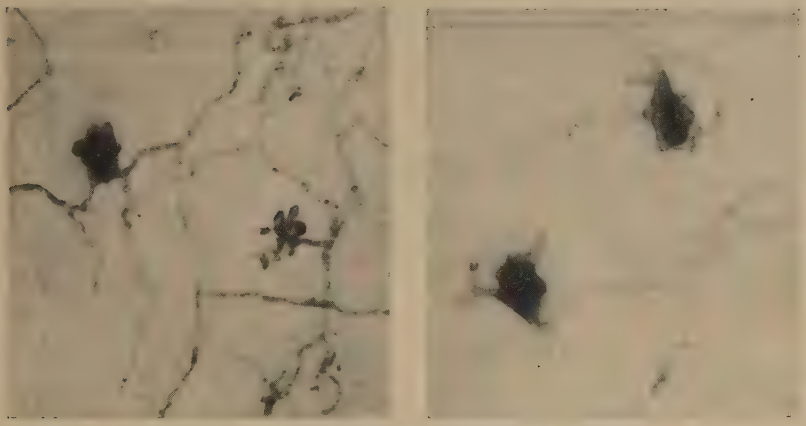

Fig. 17.—Natural infection with *M. musae*. Mount of leaf showing first visible signs of infection (X384).

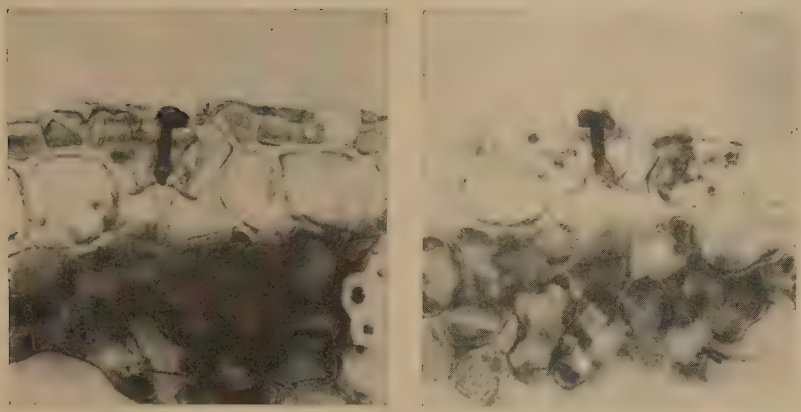

Fig. 18.—Freehand sections of banana leaf showing first stage of penetration by *M. musae* through stomatal pore (X384).

With the formation of the appressoria the epiphyllic hyphae become thicker and darker in colour. They may measure up to 3μ dia., especially close to the appressoria.

(h) Growth-Temperature Relationships

In addition to observations on growth-temperature relationships in culture made according to the method previously described and graphed in Figure 4, the effect of temperature on germ-tube growth was investigated. Using the

technique described above, leaf discs containing perithecial material were suspended above slides coated with plain agar. Single petri dishes were then placed in each of a number of chambers in the multi-temperature range incubator to give a number of temperature regimens ranging from 12°C to 37°C. The dishes were incubated for three days and at the end of this period the slides were flooded with cotton blue, covered with oblong coverslips and examined. Fifty germ-tubes were measured on each slide. The length was expressed in microns.

Two such experiments were conducted and the results are shown in graph form in Figure 19.

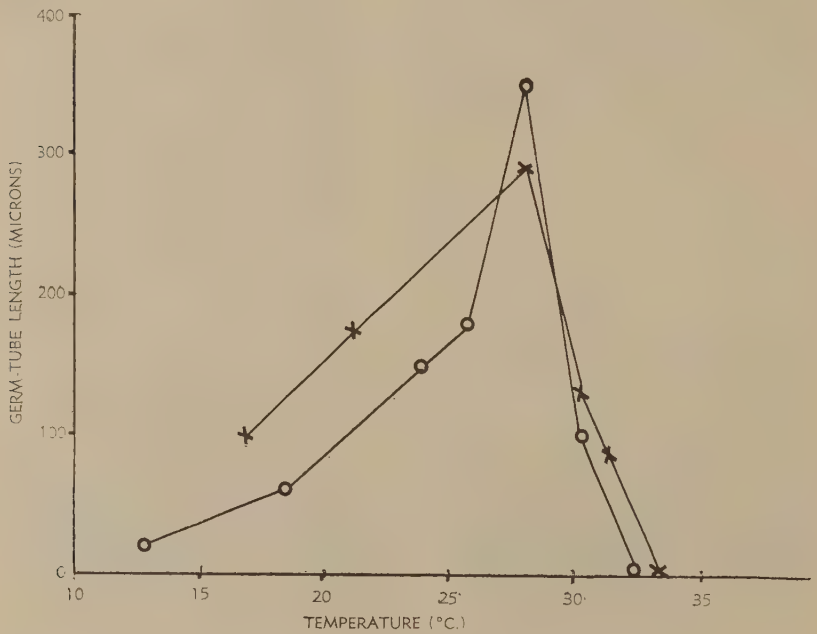

Fig. 19.—*Mycosphaerella musae*. Effect of temperature on germ-tube development. Three days' incubation on potato dextrose agar.

Examination of Figure 4 shows that while the optimum temperature was close to 26°C (79°F) the fungus grew well over the range 20°–30°C (68°–86°F). Growth was negligible above 33°C and below 10°C. The optimum temperature for germ-tube growth was in the vicinity of 27°C. There was no spore germination below 12°C or above 32°C, although ascospores were ejected from some of the leaf discs incubated at temperatures beyond these limits. The lack of germination in these cases may, however, have been due to belated discharge of the spores.

A comparison of the growth-temperature curve for *Mycosphaerella musae* (Speg.) Syd. with that for *M. musicola* Leach as determined by Simmonds (1933) (Figure 4) shows that the temperature requirements of these two species are very similar.

(i) Identity of the Speckle Fungus

The immersed, aparaphysate perithecia and the 2-celled, hyaline, cylindrical or obtuse fusoid ascospores place the banana leaf speckle fungus within the genus *Mycosphaerella.*

Several Mycosphaerella species have been recorded on the banana plant. *Sphaerella musae* Speg. was first described by Spegazzini (1909, p. 354). associated with an indefinite spotting on decaying foliage of *Musa sapientum* in Argentine. Spegazzini's description of the symptoms—"maculae quandoque plane nullae quandoque subparvae cinerascentes indeterminatae"—is significant in the present connexion.

H. & P. Sydow (1914) recorded *Mycosphaerella musae* (Speg.) Syd. on dying leaves of *Musa sapientum* in the Philippines. They were apparently somewhat doubtful of the identity of the Philippine material with Spegazzini's species, as the asci and ascospores were both considerably broader. (Measurements kindly made by E. W. Mason from one collection of Philippine material held by the Commonwealth Mycological Institute were similar.) Later, however, Saccardo (1917) described *Sphaerella musae* Sacc. on dying stems of *Musa coccinea* from the Philippines. The spots were "vagis, expallescentibus, indeterminatis" and the asci and ascospore measurements more closely resembled Spegazzini's description.

Reinking (1918), in discussing Philippine plant diseases, described and illustrated a leaf spot on *Musa sapientum* and *Musa textilis* Nee ascribed to *Mycosphaerella musae* Speg. No spore measurements were given and the illustration suggests a circumscribed spotting only rarely seen in the Queensland disease.

Sphaerella musae Speg. was recorded again from South America on living banana leaves by Ciferri and Fragoso (1927).

Stahel (1937) found perithecia of a fungus he described as *Mycosphaerella minima* in association with other fungal flora on the dry centres of Cercospora leaf spots (*Cercospora musae* Zimm.) on bananas in Surinam. *Mycosphaerella musicola* Leach, the perfect stage of *Cercospora musae* Zimm., was later identified in Jamaica (Leach 1941) and has since been found in other countries, including Queensland (Figure 15).

In Table 3 the dimensions recorded for fruiting structures of the various species of *Mycosphaerella* mentioned above are presented. When these comparative data are considered it appears that the Queensland organism could well be referred to Spegazzini's species. This evidence is substantiated further by the fact that the Queensland *Mycosphaerella* is not associated with margined leaf spots and in this agrees with Spegazzini's description. In this these two fungi differ from the other *Mycosphaerella* species recorded on banana with the exception of *Sphaerella musae* Sacc. and there appears to be justification for assuming that *Sphaerella musae* Sacc. is synonymous with *Sphaerella musae* Speg. There is still uncertainty regarding some of the Philippine records of this species.

The tentative identification of the Queensland speckle fungus was confirmed by E. W. Mason of the Commonwealth Mycological Institute, who advised that as the data established for the Queensland species completely covered Spegazzini's original description he was disposing of the fungus as *Mycosphaerella musae* (Speg.) Syd.

The conidial fungus *Phyllosticta musa-sapientii* Frag. et Cif. was recorded in association with *Sphaerella musae* Speg. on flaccid or dry leaves of *Musa sapientum* in Santo Domingo (Ciferri and Fragoso 1927). The spores of this fungus were described as hyaline, cylindrical, sub-bacillar and 3–4.5μ, x 1–1.3μ. The pycnidia were 60–110μ in diameter.

During the investigations reported herein it was found that dense clusters of small spore-like bodies could always be obtained together with *Mycosphaerella* ascospores from dead speckle-infected banana leaves (Figure 20). The shape and size of these bodies were variable. They ranged from obtuse oblong rods measuring 4–5μ x 1–2μ to sub-globose spores with a diameter of 5–6μ. The rod-shaped cells were biguttulate. On odd occasions small flask-shaped or depressed globose sacs of the same approximate size as perithecia (approx. 80μ dia.) were seen in sections of diseased leaves, but only on the upper surfaces. These contained dense masses of irregularly oblong bodies measuring no more than 4μ in length. While these structures bore some resemblance to the pycnidia and conidia of *Phyllosticta musa-sapientii* Frag. et Cif., germination was never observed on plain agar under conditions which resulted in good germination and germ-tube growth of ascospores. It has therefore been assumed that they were spermatia and spermogonia.

Some basis for this assumption has been provided by the remarks of Wolf and Wolf (1947): "Some species do not possess conidial stages, for example,

Fig. 20.—Group of spermatia and germinating ascospores on plain agar. (X384).

Mycosphaerella fraxinicola [Wolf (1939)] and *M. nyssaecola* [Wolf (1940, 1940a)]. *Mycosphaerella fraxinicola* is associated with *Phyllosticta viridis* and *M. nyssaecola* with *P. nyssae;* both of these species of Phyllosticta proved to be not conidial stages but spermogonial stages".

(j) Dissemination and Infection Site

The ascospores are airborne and are emitted when leaf tissue containing perithecia is wetted. It has been proved by inoculations (see above) and by experiments with fungicides (see below), in which comparisons were made of the control obtained by spray treatment of upper and lower leaf surfaces, that infection originates on the under-side of a leaf. Inoculations have shown that leaves older than the first expanded leaf can be infected and this is evidently quite common under natural conditions. However, examination of leaves on plants exhibiting speckle lesions on living and dead lower leaves always shows a large number of spores of *M. musae,* germinated or otherwise, on the first leaf and it has been assumed that some of the infection on a plant can originate from spores which alighted on this leaf.

(k) Epidemiology

Unlike Cercospora leaf spot, the development of this disease is not inhibited by shading. In North Queensland it is the usual case that if leaf spot is present in a plantation Mycosphaerella speckle is relatively inconspicuous. This is due to the prolonged incubation period of the latter disease and the masking of its symptoms by those of the more virulent leaf spot. However, when plantations are situated within rain-forest, Mycosphaerella speckle, together with those caused by *Chloridium musae* and *Ramichloridium musae,* is often quite noticeable on plants around the edges in the shelter of the surrounding trees, where *Mycosphaerella musicola* does not thrive. As a general rule the speckle diseases are always more prevalent in sheltered spots or in hollows or wet spots in a plantation, that is, where air movement is at a minimum and humidity is high. Mycosphaerella speckle has been observed to behave similarly in South Queensland (Simmonds 1933). In contrast to the tropical speckles, however, *M. musae* can develop quite well in exposed situations.

The factors which appear to influence speckle development are considered below.

(i) Humidity

The influence of high atmospheric humidity is quite evident under natural conditions during rain periods or early in the morning following heavy dews when a rapid watersoaking of infected tissue precedes the appearance of new lesions or extension of existing lesions. The effect of favourable humidity was shown experimentally by the results of the infection experiment (inoculated March 22, 1960) summarized in Table 2. Typical speckle lesions were reproduced within 45 days on two of the leaves inoculated on the lower surface

and enclosed each night for the duration of the experiment in a saturated atmosphere. This is much quicker development than has been observed to occur without saturation, e.g. speckle lesions from the successful inoculation made on November 7, 1956, did not show until 102 days later; while the minimum incubation period recorded (inoculated December 13, 1956) was 80 days.

(ii) Temperature

The temperature relationships of the causal fungus have been previously commented on. It is plain that as temperatures fall below 70° F there is an increasing retarding effect on the development of the disease.

(iii) Plant Vigour

Leaf damage is always more severe on backward plants. This may be because the growth rate of the plant as measured by the rate of extrusion of leaves determines the relative amount of damage on a plant. While the host is growing quickly, leaf development keeps ahead of the disease. When the growth rate is slow, the effects of speckle infection are more evident because the ratio of healthy leaf surface to speckled leaf surface is decreased due to the slower rate of leaf extrusion. However, there may also be physiological differences in backward plants which accelerate the rate of development of the disease.

(iv) Leaf Senescence

When the senescence of a leaf is accelerated by removing it from the plant, speckle development is also accelerated. This is particularly noticeable if a young leaf not showing visible speckle symptoms is removed from a speckle-infected plant and kept indoors for several days. The growth of the fungus is expedited so much under these circumstances that it is usual for lesions to appear within two or three days of removal. On vigorous plants speckle damage is confined to the basal leaves and serious defoliation does not occur until after vegetative growth has ceased. In the course of the inoculation experiments it was noted that when young leaves were inoculated the disease developed within about six weeks to the brown blotch stage; development was then apparently suspended until the inoculated leaf was well down the plant.

(v) Injury

Speckle development on a leaf often shows first around an injury, e.g. where the lamina has been torn by wind or some other agency. The results of the inoculations made on January 29, 1960 (Table 2) suggest that damage to the inoculation site in the form of pin pricks has resulted in quicker development of speckle. It seems possible, therefore, that injury resulting from red spider infestation may expedite the development of speckle lesions.

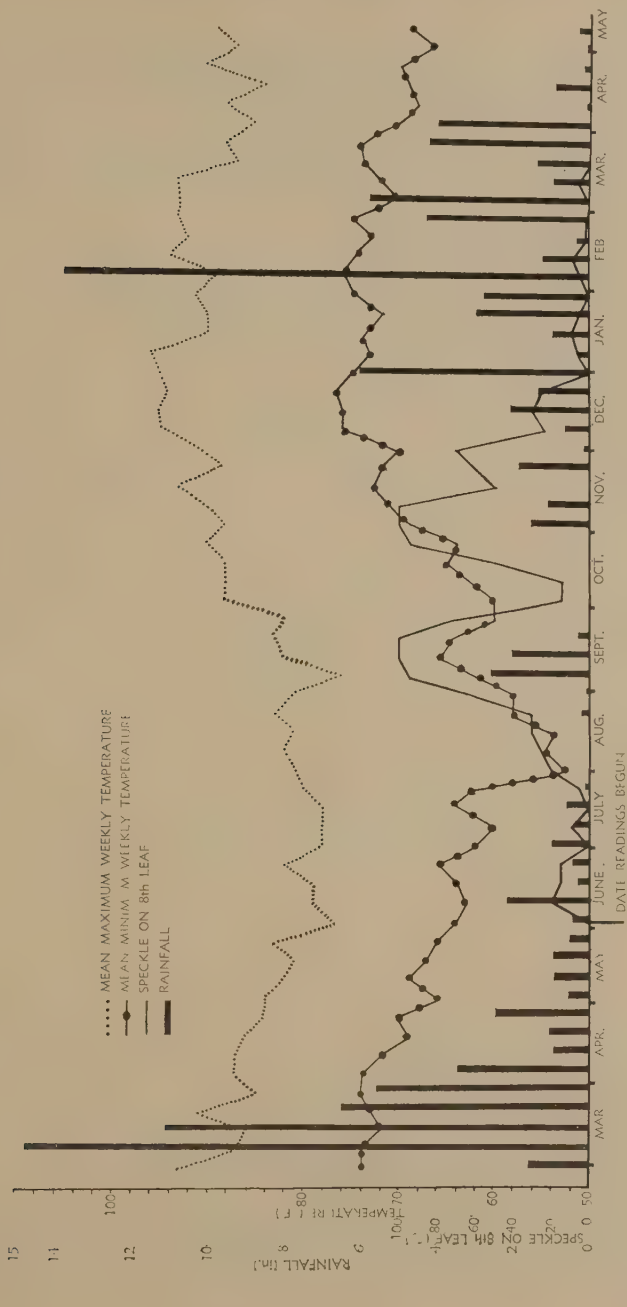

Fig. 21.—*Mycosphaerella mitsac*. Weekly record of amount of infection on the eighth expanded leaf of Ducasses plant, Kamerunga, 1959-60.

(vi) Interaction

In North Queensland, Mycosphaerella speckle is present and causes leaf loss throughout the year. There is, as would be expected, a noticeable build-up during the wet season in the late summer and autumn. However, growth rate of the plant is also at a maximum at this time of the year due to the favourable environment, and damage is usually relatively no greater than during the dry cool weather in late winter and spring when the plants are growing very slowly.

This is illustrated in Figure 21 by plotting the percentage leaf surface affected by speckle each week on the 8th fully expanded leaf at that time on a Ducasses plant growing in the open at Kamerunga, near Cairns. Mean weekly maximum and minimum temperatures and the weekly rainfalls during the period are also plotted.

The growth rate of the observation plant was very slow during late July, August and early September due to cool, dry weather during July and August. The amount of speckle showing on the 8th leaf rose from July onwards and reached a maximum in September. Due to the September rain and higher temperatures the growth rate increased and the amount of speckle damage on the 8th leaf dropped rapidly. It rose again in late October and early November when the growth of the plant once again slackened following dry, cool weather in late September and October. In December, plant growth quickened due to higher temperatures and improved rainfall and the speckle damage on the 8th leaf at that time was considerably reduced. A low incidence of speckle on the 8th leaf was sustained during January, February, March and April while the plant was putting on new leaves at a much faster rate.

These observations indicate that the inhibiting effects of low temperatures and dry weather on speckle development are offset during the North Queensland dry season by the increased susceptibility of the senescent basal leaves.

(l) Control

(i) General

In New South Wales, spray treatment each month from early December till March with copper oxychloride (1½ lb to 40 gal) plus wettable sulphur (1 lb to 40 gal) was recommended for the control of leaf spot and speckle (Anon. 1945). The sulphur was presumably included for the control of the red spider mite.

Experimental work in North Queensland later showed that spraying with copper oxychloride gave control of both diseases equal to that obtained with copper plus sulphur and a control schedule consisting of monthly applications of copper oxychloride (1½ lb to 40 gal) plus a wetting agent was recommended (Anon. 1953).

Further work in North Queensland led to the recommendation of either copper fungicides such as Bordeaux mixture (3-2-40) and copper oxychloride (1½ lb-40 gal) or zineb (1½ lb-40 gal) plus white oil (1-160) and malachite green (1-10,000) for leaf spot control (Pont 1960). In experimental work these mixtures were applied at intervals of four weeks as high-volume sprays (pressure 250 lb/sq. in.; dosage up to 200 gal/ac) to the lower surfaces of the young foliage and controlled speckle in addition to leaf spot.

While the Departmental recommendations for leaf spot control were being adopted by North Queensland growers, a modified method of application, employing reduced volume (dosage 50–60 gal/ac) without increased concentration (that is, 2 lb fungicide per acre), was introduced in many cases in order to expedite treatment, particularly on large plantations. In this method the mixture was misted down on to the plants from towers towed behind tractors. Good leaf spot control was obtained in this fashion but control of Mycosphaerella speckle was far from satisfactory.

Experiments in North Queensland with low-volume application (2 gal/ac or less) of copper oxychloride/oil mixtures to bananas showed that reasonable control of leaf spot could be obtained with this method of treatment but here again speckle control was inadequate (Pont 1960).

In New South Wales, where low-volume misting of bananas with oils is recommended for leaf spot and speckle control, oils alone were reported to be ineffective in the control of speckle (Anon. 1958, p. 21) and the inclusion of oil-miscible copper fungicides or zineb with oils also failed to give consistent control of the disease (Anon. 1959, p. 26). Recent New South Wales recommendations advocate the use of fungicide/oil mixtures in districts where speckle is usually a serious problem but some uncertainty evidently exists as to the degree of control which can be expected (Blake 1960).

(ii) Copper Residues in Relation to Speckle Control

The failure of overhead spraying and misting or fogging with copper oxychloride/oil mixtures to control Mycosphaerella speckle in North Queensland was considered to be due to an insufficient deposit of copper on the lower surfaces of older, still susceptible foliage. The following experiment was therefore carried out to compare the copper deposits immediately after the application of these various treatments. The residues after a period of weathering were also determined. The high-volume treatment of the lower surfaces of young foliage

which had been shown in experimental work to control speckle was included as a standard. The methods of application, dosage and formulae were as follows:

Treatment	Gal/Ac	Copper Oxychloride	Water	Oil
		(lb)	(gal)	(gal)
(*a*) High-pressure, high-volume spraying of undersides of heart-leaves. ("Edgell" adjustable nozzle)	200	1½	40	..
(*b*) Overhead spraying (Twin "Rega Cyclone" nozzle)	50–60	1½	40	..
(*c*) Low-volume misting ("Swingfog S.N.6." Misting attachment, 1·1 Flow Control Jet) ..	2	6½	..	5½
(*d*) Low-volume fogging ("Swingfog S.N.6." Fogging attachment, 1·0 Flow Control Jet) ..	¾–1	12	..	4

The residual copper on the leaf was analysed by the Chemical Laboratory of the Division of Plant Industry, using the following series:

(1) First expanded leaf; sampled immediately after treatment. Air-dried.

(2) Fifth expanded leaf; sampled immediately after treatment. Air-dried.

(3) First expanded leaf at time of spraying, sampled later when in fifth leaf position. Air-dried.

(4) Similar to (3), only prepared so that the deposits on upper and lower leaf surfaces could be analysed separately. Green leaf samples. Surfaces washed with 2N H_2SO_4 and worked with a small brush to remove deposited copper.

In all cases the samples were obtained by cutting the leaves into discs 9·2 cm in diameter. Treatments were replicated six times. Each plot consisted of a single leaf.

The spray deposit on leaves in Series 3 and 4 was exposed to weathering for 8 weeks, during which time 0·69 in. of rain fell on 7 wet days. Heavy dews were frequent.

The results are summarized in Table 4.

TABLE 4

Copper Residues Analyses (Mean Values)

Treatment	Total Leaf Cu (p.p.m.)			μg Cu per sq. cm leaf	
	Series 1 Leaf 1. Immediate	Series 2 Leaf 5. Immediate	Series 3 Leaf 1. After 8 weeks	Series 4 Leaf 1. After 8 weeks	
				Upper	Lower
(*a*) High-volume, Heart-leaf	2,117	563	997	2·98	10·18
(*b*) Reduced Volume, Overhead ..	711	853	247	1·13	0·45
(*c*) Low-volume, Misting	217	179	50	0·12	0·08
(*d*) Low-volume, Fogging	82	108	60	0·08	0·03

The far greater fungitoxic residue provided by high-pressure spraying with the jet directed at the lower surfaces of the youngest leaves was apparent in the means for leaf copper for Series 1. The Series 2 figures showed that overhead spraying gave a greater residue on what was the fifth leaf at time of spraying. However, this would have been almost entirely located on the upper surface of the leaf, where its value for speckle control would have been negligible. The value of 563 p.p.m. leaf copper for high-volume heart-leaf spraying in this series indicated that fall-out during spraying deposited more copper on the leaf than did either misting or fogging with oil-based concentrate.

The amount of copper remaining on the first leaf after 8 weeks' exposure (Series 3) was far greater for the high-volume treatment than for any of the other three and the detailed analyses (Series 4) revealed that over three times as much copper remained on the lower leaf surface as on the upper. The amount of copper recovered from the lower surfaces of leaves which had been overhead sprayed, misted or fogged and similarly exposed for 8 weeks was negligible in comparison. In the case of these three treatments there was more residue on the upper surface of the leaf than on the lower.

It appears therefore that the unsatisfactory control of speckle obtained with the last three methods of application may be due to the fact that the amount of fungicide deposited on the under-sides of the older leaves is negligible. In addition, the fungitoxic residues on the lower surfaces of the youngest leaves are reduced by weathering to such an extent that infection by the speckle fungus at a later date when the leaf is further down the plant is not prevented.

(iii) Experiment with Protectants

The success of a mixture containing copper oxychloride, white oil emulsion and malachite green for banana leaf spot control led to an investigation of the control of *Mycosphaerella musicola* obtained with the ingredients of this mixture

used either singly or in pairs. This work showed that white oil emulsion alone and white oil emulsion/malachite green controlled the disease equally as well as the complete mixture (Pont 1960). It was necessary to know the efficacy of these sprays against *Mycosphaerella musae* and this aspect of their fungitoxicity was investigated in a series of screening trials conducted during 1958 and 1959. The fungicides zineb and phenyl mercuric chloride and the misting oil "Mobil Orchard Oil No. 1," which is typical of a large number of similar products with high unsulphonatable residue being used in Australia and overseas for leaf spot control, were also screened in these trials.

Plants of the variety Ducasses were employed in these tests because of their immunity to leaf spot (*M. musicola*). Details of procedure are given below.

(1) *Trial 1.*—The youngest fully expanded leaf on each plant was sprayed and each week for 10 weeks any freshly expanded leaves were treated. The results were based on the amount of infection which subsequently appeared on these leaves until the time of their death. Single-plant plots were used and there were three replications of four treatments.

(2) *Trial 2.*—The four youngest fully expanded leaves on each of two plants were treated. The treatments were applied with a nebulizer to circles 4·5 cm in diameter marked on the leaf surface with a "Chinagraph" pencil. The five treatments employed were replicated five times on each of the four treated leaves on each plant, giving 10 replications for each leaf position.

(3) *Trial 3.*—The sprays were applied to circles as before located on the five youngest expanded leaves on each of four plants. The treatments were replicated twice on each leaf to give 8 replications of 6 treatments for each leaf position.

(4) *Trial 4.*—The treated circles were randomized on each of the six youngest expanded leaves of four plants. Treatments were again applied with a nebulizer and were replicated twice on each leaf to give 8 replications of 4 treatments for each leaf position.

The efficacy of the treatments was assessed by a visual appraisal of the percentage of the surface infected with speckle on a sprayed leaf or within a treated circle and from these data means for each treatment were calculated. The results are summarized in Table 5.

A single application of either copper oxychloride or copper oxychloride/white oil emulsion/malachite green on the lower surface gave good control of speckle, and copper oxychloride was efficacious even when the fifth and sixth leaves were treated. Zineb also was very effective. White oil emulsion was slightly inferior to these two fungicides. Phenyl mercuric chloride was inferior to copper oxychloride and was roughly as effective as "Mobil

TABLE 5

Combined Results from Fungicide Screening Trials
(Mean Percentage Speckle Infection)

Trial No.	Treatment	Leaf Position when Treated and Surface											
		1		2		3		4		5		6	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
1	Copper oxychloride (1½–40)	0·6	74										
	White oil emulsion (1–160)/malachite green (1–10,000)	9·3											
	Untreated	94	94										
2	Copper oxychloride (1½–40)/white oil emulsion (1–160)/ malachite green (1–10,000)	1		0		0		10					
	White oil emulsion (1–160)/malachite green (1–10,000)	2		0		1		22					
	White oil emulsion (1–160)	1·5	25	3	18	9	53	38	71				
	Untreated	26	26	16	16	51	51	83	83				
3	Copper oxychloride (1½–40)	0		0·7		0·2		8·3		36·2			
	Zineb (1½–40)	4·3		7·1		0·1		7·8		47·5			
	White oil emulsion (1–160)	2·8	43	12·7	45·1	1·6	54·3	20·7	90·6	76·2	86·2		
	Malachite green (1–10,000)	28·1		27·1		8·3		50·0		87·5			
	White oil emulsion (1–160)/malachite green (1–10,000)	3·4		13·8		1·5		25·6		75·6			
	Untreated	57·5	57·5	62·0	62·0	77·5	77·5	90	90	91·8	91·8		
4	Phenyl mercuric chloride (0·1%)	27·5		25·7		1·3		15·8		14·5		10	
	Copper oxychloride (1½–40)	0		0		0		0·7		2·1		2·7	
	" Mobil Orchard Oil No. 1 "	5		25·6		4·8		22·6		30·8		27·5	
	Untreated	59·3		58·1		93·1		80·6		90·6		90·6	

Orchard Oil No. 1," Malachite green was worse than any of the above sprays and the efficacy of a white oil emulsion/malachite green mixture was equivalent to that of the white oil alone.

Both copper oxychloride and white oil emulsion were ineffective when applied to the upper surface.

The efficacy of copper oxychloride when applied to the fourth, fifth and sixth leaves is of particular interest as it indicates that effective penetration of the fungus had not been obtained at this stage.

(iv) Experiments with Eradicants

Phenyl mercuric chloride (0·1 per cent.) was shown to prevent the development of perithecia in apple leaves infected with *Venturia inaequalis* (Hutton 1954) and a pre-leaf-fall eradicant spray with this chemical was later found to control the disease (Anon. 1956).

Pentachlorphenol (0·75—0·2 per cent.) has recently been found to prevent ascospore discharge from mature perithecia of *Mycosphaerella pinodes* on pea straw (Carter 1959). The contents of the perithecia were completely destroyed by this treatment. Phenyl mercuric chloride (0·1 per cent.) failed to destroy mature perithecia of this fungus.

The evergreen habit of the banana plant when considered in relation to the fact that the leaf speckle disease is active throughout the year places limits on the practicability of eradicant spraying for the control of *Mycosphaerella musae*. Nevertheless, two possibilities appear to exist, viz. (1) regular spraying with a fungicide which has eradicant as well as protectant properties, and (2) regular detrashing followed by ground spraying as an adjunct to the usual schedule of protectant sprays. In many instances normal plantation management calls for the regular application of weedicides and it would be preferable if the chemical employed as a ground spray had eradicant as well as weedicidal properties.

Some preliminary trials were undertaken as a first step in the assessment of the feasibility of eradicant spraying for banana leaf speckle control. These are discussed below.

(1) *Trial* 1.—This was designed to investigate the effect of phenyl mercuric chloride on the development of perithecia. Heavily infected leaves on Ducasses plants were sprayed on both surfaces with phenyl mercuric chloride (0·1 per cent.) while still alive and prior to the formation of perithecia. When the leaves were dead, leaf discs were cut from them and from comparable untreated leaves. These discs were placed on damp cotton wool in the lids of 4 in. petri dishes and exposed for three days at 80°F over ten $\frac{7}{8}$ in. cover slips located in the bottom of the dishes. Seven dishes were allowed for each treatment. Three cover slips were selected at random from each of the seven dishes for examination. They

were mounted on slides and scanned with the low-power objective of a microscope. Ratings were given for density of ascospore discharge using the following scale:

1—Spores in scattered groups with a density not exceeding 10 spores within the area bounded by the length and breadth of a micrometer eyepiece scale (approx. 0·18 sq. mm).

2—Scattered groups. Density 11—20 spores.

3—Scattered groups. Density 21—30 spores.

4—Spores in aggregated groups. Density 31—40 spores.

5—Aggregated groups. Density 41—50 spores.

The mean values for the ratings for the two treatments were phenyl mercuric chloride (0·1 per cent.) 0·4, untreated 1·5.

(2) *Trial* 2.—The object of this experiment was to determine the effect of chemical treatment on ascospore discharge from mature perithecia. Dead leaves from Ducasses plants were sprayed thoroughly on their lower surfaces. Selected circular areas smaller in diameter than a $\frac{7}{8}$ in. coverslip and containing dense aggregations of perithecia were excised, pinned to small pieces of blotting paper and fixed in position on moist cotton wool in the lids of 4 in. petri dishes so that each leaf circle was directly over a coverslip in the bottom of the dish. The five treatments were randomized in each of five dishes. After three days' incubation at 80°F the coverslips were removed and rated for ascospore density as above.

Treatments and results were as follows:

Treatment	Mean Rating
Copper oxychloride (1½–40)	3·2
Phenyl mercuric chloride (0·1 per cent.)	3·2
Malachite green (1–10,000)	2·4
White oil emulsion (1–160)	2·8
Unsprayed	3·2

(3) *Trial* 3.—The main purpose of this trial was an investigation of the eradicant properties of sodium pentachlorphenate, which is a widely used weedicide. Phenyl mercuric chloride was included for comparison purposes and a new eradicant spray Cyanamid "Cyprex" (65 per cent. n-dodecyl guanidine acetate) was also tested.

Leaf discs containing mature perithecia were selected from dead leaves on Ducasses plants, sprayed on their lower surfaces, dried and then fitted into the lids of petri dishes on damp cotton wool with their lower surfaces exposed. There were six replications of each treatment.

The plates were incubated for three days at 80°F. At the conclusion of this period the bottoms of the dishes were examined with the low-power objective of a microscope and spore counts were made. In doing this two traverses of each plate were made along diameters at right angles. At seven points along each traverse the numbers of spores contained within the length and breadth of a micrometer eyepiece scale were counted. Treatments and results were as follows:—

Treatment	Mean Spore Count
Sodium pentachlorphenate (0·2 per cent.) ..	0
"Cyprex" (8 lb–100 gal)	1
Phenyl mercuric chloride (0·1 per cent.)	7·2
Unsprayed	12·8

(4) *Trial* 4.—In this experiment the eradicant properties of sodium pentachlorphenate were compared with those of an arsenical weedicide (56 per cent. w/v As_2O_3) commonly employed in banana plantations in Queensland. The effect of treatment of upper *v.* lower leaf surface was also investigated. The procedure followed was similar to that used in Trial 3. Leaf discs from dead speckled leaves of the Cavendish variety were treated. The lower surfaces of the treated leaf discs were exposed in all cases. There were four replications of each treatment. The petri dishes containing the leaf discs were incubated for 24 hr at 80°F.

This experiment was repeated in Trial 5, using five replications of the same treatments. The results of both trials are given below:

Treatment	Mean Spore Count	
	Trial 4	Trial 5
Sodium pentachlorphenate (0·2 per cent.)—		
Lower surface	0·1	0
Upper surface	11·8	0·8
Arsenical weedicide (1 gal–50 gal)—		
Lower surface	3·3	0·4
Upper surface	5·0	1·7
Unsprayed	13·2	6·8

The indications from these trials were that phenyl mercuric chloride inhibited subsequent ascospore discharge when leaves in which the fruiting structures had not developed were treated, but had much less effect on spore discharge from leaves in which the perithecia were mature when the spray was applied. Sodium pentachlorphenate almost completely inhibited ascospore discharge from mature perithecia when sprayed onto the lower leaf surface. "Cyprex" was also effective in this respect but it should be borne

in mind that the concentration employed, viz. 0·52 per cent. active ingredient, was 10 times as strong as that normally employed as an eradicant spray for apple black spot control.

Treatment of the upper leaf surface with sodium pentachlorphenate was not so effective. The majority of the perithecia of *Mycosphaerella musae* open to the lower leaf surface. These results suggest, therefore, that the chemical or its vapour penetrates into the perithecia through the ostioles.

An indefinite result was obtained from treatment of either leaf surface with are arsenical weedicide at the concentration employed, i.e. normal weedicidal strength.

(v) Conclusions

In this experimental work a single application of a standard fungicide to the under-side of a leaf gave complete and lasting freedom from speckle attack to that leaf. This treatment was effective on all leaves not showing visible infection when the spray was applied. The dosage used would approximate to that employed when high-volume spray treatment of a banana plantation is practised, i.e. around 6 lb fungicide per acre on plants nearing flowering. These results suggest that the method of overhead spraying at intervals of four weeks with copper oxychloride/white oil/malachite green mixture, which has failed to give effective control of speckle in North Queensland plantations, can be modified by alternating at intervals of four weeks an overhead spray for leaf spot control and a combined leaf spot and speckle control schedule in the form of high-volume treatment directed at the under-sides of the leaves.

Manual overhead spraying for leaf spot control is, however, now being displaced in North Queensland by automatic treatment, employing air-blast misting machines towed behind tractors. Dosage approximates 60 gal per ac. With this method, coverage of the under-sides of all leaves is possible and the amount of fungicide applied—2 lb per ac—appears to persist sufficiently long to permit of good speckle control if the treatment is repeated at intervals of four weeks. Some growers aim to treat their plantations at intervals of three weeks during the wet season. Treatment of a plant has to be continued until the bunch is taken off.

The fact that the disease is disseminated by ascospores ejected from dead leaves indicates that detrashing should play a big part in the control of speckle. Periodic removal and destruction of the dead leaves draped around the base of a stalk would undoubtedly drastically reduce the amount of inoculum available and increase the efficacy of the spray programme.

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EFFECTS OF FRUIT FORM REMOVALS ON COTTON YIELDS IN CENTRAL QUEENSLAND

By T. PASSLOW, M.Sc.Agr.,* and K. G. TRUDGIAN, B.Sc.†

SUMMARY

During 1957-1959 four trials in both irrigated and rain-grown cotton were conducted in Central Queensland. Removals of all squares and bolls were carried out during eight weeks following the first burst of squaring, approximately three weeks after the appearance of the first squares.

Removals during the first four weeks did not affect yields. Maturing of replacement squares following removals during the next four weeks was dependent on length of season and growing conditions.

The effect of loss and damage among squares and bolls late in the season was demonstrated by an unusually severe *Heliothis armigera* (Hubn.) attack during March and the incidence of *Pectinophora scutigera* (Hold.) during 1958-59.

I. INTRODUCTION

In Central Queensland, cotton is sown from mid-October to early December. Early plantings usually give the best yields although these crops often suffer loss of squares and young bolls following either or both severe heat and insect attack during December and early January. This loss has been considered of major importance since the inception of the industry in Central Queensland, where *Heliothis armigera* (Hubn.) is the insect most commonly associated with fall of squares and bolls in December and early January. Passlow (1958, 1959), however, demonstrated that insecticide treatments for control of *H. armigera* during the early square production period had no influence on total yields although treatments increased the first pick. Second-pick yields were lower in sprayed than in untreated areas, where squares and small bolls lost to pest attack were replaced later in the season.

In the United States of America, Eaton (1931) studied plant reaction to early defloration and obtained yield increases following manual removal of early buds. Later, Dunnam, Clark, and Calhoun (1943) showed that removal of squares for periods as long as six weeks from first production had no effect on yields provided rainfall was above normal. In East Africa, McKinlay and

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Geering (1957) and Coaker (1957) found that protection from insect attack during the first four months did not affect yields in early-sown crops, but gave yield increases in late crops.

These results and those of Passlow (1958, 1959), where plant recovery occurred following relatively low loss of early squares and young bolls, suggested the present investigations in which plant reactions to complete removals of fruiting forms were studied.

Four trials were carried out. Trials 1 (1957-58) and 3 (1958-59) were established in irrigated Miller 43-9-0 at the Biloela Regional Experiment Station, and Trials 2 (1957-58) and 4 (1958-59) in rain-grown Arkot 2-1 at Gracemere.

II. METHODS

Trials 1–4 were planted on October 15, November 7, October 14 and December 8, respectively, each as a 6 x 4 randomized block with a plot size of either 25 ft (Trials 1 and 2) or 20 ft (Trials 3 and 4) of two adjacent rows, 3 ft 6 in. apart.

In Trial 1 treatments were:

A: No removal of fruit forms.

B: Weekly removal of all fruit forms for two weeks commencing November 26 at the first burst of squaring, approximately three weeks after the appearance of the first squares.

C: Weekly removal of all fruit forms for four weeks commencing November 26.

D: Weekly removal of all fruit forms for six weeks commencing November 26.

E: Weekly removal of all fruit forms for eight weeks commencing November 26.

F: No removal of fruit forms.

In Trial 2 treatments were basically similar to those in Trial 1 except that removals were commenced on January 10, one week after the first burst of squaring, and carried out for 1, 3, 5 and 7 weeks in Treatments B, C, D, and E.

In Trials 3 and 4 treatments were:

A: No removal of fruit forms.

B: Removal of all fruit forms on December 16 in Trial 3 and February 4 in Trial 4, two weeks after the first burst of squaring.

C: Removal of all fruit forms on December 30 in Trial 3 and February 19 in Trial 4, four weeks after the first burst of squaring.

D: Removal of all fruit forms on January 14 in Trial 3 and March 4 in Trial 4, six weeks after the first burst of squaring.

E: Removal of all fruit forms on January 28 in Trial 3 and March 19 in Trial 4, eight weeks after the first burst of squaring.

F: No removal of fruit forms.

In each trial, all treatments except F were sprayed to prevent damage from insects, particularly *H. armigera.* The insecticides used were:

DDT.—An emulsion concentrate containing 25 per cent. *p.p'* isomer.

Parathion.—An emulsifiable preparation containing 25 per cent. w/v active ingredient.

DDT 0·1 per cent. was applied 11 times in Trials 1 and 2 and 12 times in Trials 3 and 4 at weekly intervals commencing November 26, January 10, December 9 and January 29 respectively. Parathion 0·05 per cent. was incorporated with the applications on February 25, March 7 and March 14 in Trial 2 and on March 13 in Trial 4 to prevent build-up of the mite *Tetranychus ludeni* Zacher. Thorough coverage to run-off was achieved at all applications, using a power-driven, hand-operated, twin-nozzle unit.

Trials 1–4 were harvested weekly from February 25 to July 2, March 5 to July 2, February 24 to June 29 and April 2 to July 10 respectively.

Results were assessed in terms of numbers of fallen fruit forms, production of fruit forms and yields.

All fallen forms were collected weekly from the inter-row space in each plot; totals of squares and bolls and numbers damaged by insects were recorded. Numbers of larvae of pest species found in damaged forms in Trials 2, 3 and 4 were recorded. These were predominantly *Pectinophora scutigera* (Hold.), with some *Earias huegeli* Rog. and *Dichocrocis punctiferalis* (Guen.) and a few *H. armigera.*

Fruit form production was calculated from the data on fallen forms and weekly records of squares, bolls and mature bolls on selected plants. A square became a boll when, following fertilization, the floral parts commenced to dry out and either fell or could be readily lifted away. A boll was mature when dried out. Mature bolls were divided into pickable (those carrying at least one lock of sound cotton), and unpickable (those carrying no sound cotton).

Prior to commencement of squaring, five (Trials 1 and 2) or four (Trials 3 and 4) representative plants were selected in each plot and weekly records of numbers of squares, bolls and matured bolls on each were taken. Weekly production of forms was calculated by subtracting the number of forms on the plants at the beginning of each week from that on the plants at the end of the week, and adding to this figure the calculated number of forms which fell from an equivalent number of plants during the week. It was assumed that the number of fallen forms collected was half the total fall from plants in a plot.

Harvesting was carried out weekly. Weight of seed cotton and numbers of pickable and unpickable bolls were recorded.

III. RESULTS

Results are presented in Tables 1–12. The inverse sine was used to transform percentage damaged fallen forms. Yields are expressed in the economic unit of pounds of seed cotton per acre, and where necessary are adjusted to an equal number of plants.

IV. DISCUSSION

(a) Trial 1

Despite large differences in numbers of fallen forms and in production of forms (Tables 1 and 2), no significant yield differences (Table 3) were obtained. These results are similar to those of Passlow (1958, 1959), who obtained higher production of forms in unsprayed than in sprayed cotton without yield differences. Good growing conditions were experienced throughout the trial; under these conditions recovery from loss of early fruit forms will occur irrespective of the cause of such loss.

TABLE 1

Trial 1. Total and Damaged Fallen Forms

Treatment	Mean per Plot	Mean Damaged	Damaged Forms (%)	
			Trans. Mean	Equiv. Mean (%)
No removal, sprayed	814	104·2	21·1	13·1
Removal for 2 weeks, sprayed	826	118·2	22·3	14·4
Removal for 4 weeks, sprayed	1,013	104·0	18·5	10·0
Removal for 6 weeks, sprayed	1,643	214·2	21·2	13·1
Removal for 8 weeks, sprayed	1,570	155·0	18·3	9·9
No removal, unsprayed	1,083	234·5	27·9	21·9
Necessary differences for significance 5%	200	36·2	2·7	
Necessary differences for significance 1%	276	50·1	3·7	

TABLE 2

Trial 1. Production of Fruit Forms

Treatment	Production per 5 plants		Date of Last Removal
	Total (Nov. 26–July 2)	From Time of Last Removal	
No removal, sprayed	330	330	..
Removal for 2 weeks, sprayed	370	356	Dec. 3
Removal for 4 weeks, sprayed	462	384	Dec. 16
Removal for 6 weeks, sprayed	812	545	Jan. 2
Removal for 8 weeks, sprayed	948	538	Jan. 13
No removal, unsprayed	435	435	..
Necessary differences for significance .. 5%	66	46	
Necessary differences for significance .. 1%	91	63·9	

The much higher production of forms obtained when removals were carried out for as long as six and eight weeks shows that the potential yield was increased. At the same time, however, fallen form numbers were comparatively higher in these treatments, indicating that the yields obtained in the no-removal/sprayed plots were the maximum for the conditions prevailing during the trial.

TABLE 3

Trial 1. Yields, Numbers of Plants and Mature Bolls

Treatment	Yield (lb/ac)	Plants per Plot	Mature Bolls		
			Total	Pickable	Unpickable
No removal, sprayed	1,618	48·2	766	578	188
Removal for 2 weeks, sprayed ..	1,612	49·0	789	585	204
Removal for 4 weeks, sprayed ..	1,748	48·2	715	604	112
Removal for 6 weeks, sprayed ..	1,506	49·0	788	599	190
Removal for 8 weeks, sprayed ..	1,506	48·0	828	659	170
No removal—no spray	1,668	47·8	765	591	174
Necessary differences for significance 5%	255	3·3	71	72	44
Necessary differences for significance 1%	353	4·5	98	100	62

(b) Trial 2

Significant yield increases are associated with the increased fruit form production following removals for periods as long as five and seven weeks (Tables 4 and 6). Field conditions were poor until mid-January, when good rainfall occurred; the remainder of the season was excellent. When removals were carried out for as long as five and seven weeks the plants were forced to produce their crop during this latter period. The no-removal/sprayed treatment gave better yields than the unsprayed treatment following an Heliothis attack during March. This demonstrates that pest attacks late in the season when replacement of fallen forms is most unlikely can cause economic losses (see Passlow 1958).

TABLE 4

Trial 2. Total and Damaged Fallen Forms

Treatment		Mean per Plot	Mean Damaged	Damaged Forms (%)	
				Trans. Mean	Equiv. Mean (%)
No removal, sprayed		679	140	26·8	20·3
Removal once, sprayed		528	90	24·2	16·9
Removal 3 times, sprayed		638	82	21·1	13·0
Removal 5 times, sprayed		1,041	118	19·8	11·5
Removal 7 times, sprayed		980	141	22·4	14·5
No removal, unsprayed		612	178	32·6	29·1
Necessary differences for significance	5%	173	43	2·9	
	1%	239	59	4·0	

TABLE 5

Trial 2. Production of Fruit Forms

Treatment	Production per 5 plants		Date of Last Removal
	Total (Jan. 10–July 2)	From Time of Last Removal	
No removal, sprayed	372	372	—
Removal once, sprayed	361	361	Jan. 10
Removal 3 times, sprayed	511	414	Jan. 24
Removal 5 times, sprayed	830	568	Feb. 7
Removal 7 times, sprayed	1,243	628	Feb. 21
No removal, unsprayed	327	327	—
Necessary differences for significance 5%	159	113	
1%	220	156	

TABLE 6

Trial 2. Yields, Numbers of Plants and Mature Bolls

Treatment	Yield (lb/ac)		Plants per Plot	Mature Bolls		
	Observed	Adjusted		Total	Pickable	Unpickable
No removal, sprayed	2,074	2,075	38·0	820	752	68·0
Removal once, sprayed	1,980	1,981	38·0	732	702	30·0
Removal 3 times, sprayed	2,206	2,112	41·0	863	806	57·8
Removal 5 times, sprayed	2,671	2,688	37·5	1,054	995	59·8
Removal 7 times, sprayed	2,626	2,674	36·5	1,140	1,064	76·2
No removal, unsprayed	1,606	1,631	37·2	730	634	96·5
Necessary differences for significance 5%	284		4·7	127	112	26·9
1%	393		6·5	175	154	37·2

TABLE 7

Trial 3. Total and Damaged Fallen Forms

Treatmen	Mean from Time of Removal	Damaged Forms (%)	
		Trans. Mean	Equiv. Mean (%)
No removal, sprayed	673	20·5	12·2
Removal at 2 weeks, sprayed	617	16·2	7·8
Removal at 4 weeks, sprayed	501	13·1	5·1
Removal at 6 weeks, sprayed	810	13·6	5·6
Removal at 8 weeks, sprayed	562	22·6	14·8
No removal, unsprayed	762	27·2	20·9
Necessary differences for significance { 5%	151	2·4	
1%	209	3·3	

(c) Trial 3

Growing conditions were good until early March, after which further irrigation of the trial site was impracticable and dry weather did not allow the plants on which removal was carried out six weeks after first burst of squaring to mature a high percentage of the considerable form production obtained (Table 8). The same conditions prevented high production where removal was carried out at eight weeks. These results indicate that with severe loss of forms in mid-season (removal at six and eight weeks) followed by poor growing conditions plant recovery is not good.

The unsprayed treatment produced more forms than the no-removal/sprayed treatment, as could be expected; yield, however, was lower. More bolls were harvested from the unsprayed treatment, many being partly damaged, a higher percentage of mature bolls was unpickable (Table 9) and a higher percentage of fallen forms was damaged by insect attack (Table 7) than in the no-removal/sprayed treatment. These facts and observations prove that late

TABLE 8

Trial 3. Production of Fruit Forms

Treatment	Production per Plant		Date of Removal
	Total	From Time of Removal	
No removal, sprayed	27·1	27·1	—
Removal at 2 weeks, sprayed	46·6	30·3	Dec. 16
Removal at 4 weeks, sprayed	44·2	22·6	Dec. 30
Removal at 6 weeks, sprayed	65·2	31·5	Jan. 14
Removal at 8 weeks, sprayed	47·3	21·4	Jan. 28
No removal, unsprayed	33·4	33·4	—
Necessary differences for significance .. .. .. { 5%	8·6	6·3	
1%	11·9	8·7	

attack by *Pectinophora scutigera* (Hold.) caused lower yields where no spray was applied. It is again demonstrated, as in Trial 2, that late-season pest attack can cause economic losses.

TABLE 9

Trial 3. Yields, Numbers of Plants and Mature Bolls

Treatment	Yield (lb./ac)	Plants per Plot	Mature Bolls		
			Pickable	Unpickable (%)	
				Trans. Mean	Equiv. Mean (%)
No removal, sprayed	1,980	80·5	582	7·6	1·8
Removal at 2 weeks, sprayed	1,980	78·2	612	3·3	0·3
Removal at 4 weeks, sprayed	1,639	80·5	558	6·6	1·3
Removal at 6 weeks, sprayed	998	77·2	377	7·8	1·8
Removal at 8 weeks, sprayed	608	80·8	298	13·5	5·4
No removal, unsprayed	1,668	79·0	631	14·6	6·3
Necessary differences for significance 5%	273	No sig.	89	3·4	
Necessary differences for significance 1%	377	diff.	123	4·7	

(d) Trial 4

Growing conditions were good until early March, after which dry conditions prevailed, and, as in Trial 3, despite high production of forms (Table 11) yields (Table 12) were low in plots where removal was carried out at six and eight weeks after the first burst of squaring. It is again demonstrated that recovery is poor after severe loss of forms during mid-season followed by adverse growing conditions. In addition, treatments where removals were made at six and eight weeks and the unsprayed treatment were severely damaged by *P. scutigera* late in the season.

TABLE 10

Trial 4. Total and Damaged Fallen Forms

Treatment	Mean from Time of Removal	Damaged Forms (%)	
		Trans. Mean	Equiv. Mean (%)
No removal, sprayed	463	19·9	11·6
Removal at 2 weeks, sprayed	554	23·4	15·8
Removal at 4 weeks, sprayed	612	27·0	20·6
Removal at 6 weeks, sprayed	914	26·0	19·1
Removal at 8 weeks, sprayed	502	36·6	35·6
No removal, unsprayed	637	33·8	33·8
Necessary differences for significance 5%	158	4·3	
Necessary differences for significance 1%	219	6·0	

TABLE 11

Trial 4. Production of Fruit Forms

Treatment	Production per Plant		Date of Removal
	Total	From Time of Removal	
No removal, sprayed	16·0	16·0	—
Removal at 2 weeks, sprayed	24·8	19·7	Feb. 4
Removal at 4 weeks, sprayed	36·4	22·7	Feb. 19
Removal at 6 weeks, sprayed	40·8	25·0	Mar. 4
Removal at 8 weeks, sprayed	31·0	15·6	Mar. 19
No removal, unsprayed	18·1	18·1	—
Necessary differences for significance 5%	9·2	5·3	
1%	12·8	7·3	

TABLE 12

Trial 4. Yields, Numbers of Plants and Mature Bolls

Treatment	Yield (lb/ac)	Plants per Plot	Mature Boll	
			Pickable	Total
No removal, sprayed	1,297	86	462	493
Removal at 2 weeks, sprayed	1,224	85	475	530
Removal at 4 weeks, sprayed	1,157	81	476	556
Removal at 6 weeks, sprayed	(345)	98	(180)	(258)
Removal at 8 weeks, sprayed	(78)	95	(56)	(129)
No removal, unsprayed	758	88	334	437
Necessary differences for significance 5%	162	No sig.	79	
1%	233	diff.	113	

V. GENERAL DISCUSSION AND CONCLUSIONS

In Central Queensland, loss of fruit forms during the month after the first burst of squaring does not affect yields.

Maturing of replacement forms following removals during the second month is dependent on the length of the season and growing conditions.

Eaton (1931) in the United States of America, McKinlay and Geering (1957) in East Africa and others obtained basically similar results.

In Central Queensland, length of season is not important except with December plantings in the Callide and Dawson Valleys, where removals during the second month may not allow sufficient time for crop maturity in some seasons.

Under good growing conditions replacement of forms will occur, and increased yield may follow removals. When poor early growth is followed by

good conditions, removals during the second month will not affect yields; poor conditions late in the season, however, will prevent plant recovery. Considerable risk, therefore, is associated with severe loss of fruit forms in rain-grown cotton at any stage later than one month after the first burst of squaring.

The effects of loss and damage among fruit forms late in the season is clearly demonstrated by the unusually severe *H. armigera* attack during March in Trial 2 and the incidence of *P. scutigera* during 1958-59.

VI. ACKNOWLEDGEMENTS

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NEW SPECIES OF AGROMYZIDAE FROM QUEENSLAND

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SUMMARY

New species of Agromyzidae from Queensland are *Melanagromyza wikstroemiae, M. pisi, M. polyphyta, M. indigoferae, M. dianellae,* **and** *Haplomyza caulophaga.* **The first four have leaf-mining larvae; the last stem-mining larvae.**

The name *Melanagromyza phaseoli* **(Tryon) is discussed.**

A key is given for the species of *Melanagromyza* **in Queensland.**

MELANAGROMYZA wikstroemiae sp. nov.

(Figures 1 and 2)

Length 1·5–2·0 mm (dried specimen); entirely dark brown to black excepting calypters and pulvilli, which are pale.

Head.—Outer verticals one to one-and-a-half times length of postverticals; four pairs fronto-orbitals, three superior (dorsally directed), one inferior (inwardly directed), vertical intervals between first and second pairs and second and third approximately equal and at least twice that between third and fourth pairs. Orbital setulae dorsally directed. Vertical triangle pruinose, tapering sharply towards centre of frontal vitta, then gradually to a point extending to lunule. Frontal vitta wide (about half central eye width), dull pruinose. Arista strong, more than four times length of third antennal segment, bearing short setae throughout, and basally thickened for about one-fifth of length (plainly visible at x 100 magnification). Oral vibrissae well developed, incurved, more than twice length of setae of subcranial margin of genae. Depth of genae below eye almost as great as length of oral vibrissa. Occiput pruinose with small white setae arranged in a steep narrow V.

Thorax.—Wing typical of genus, closely resembling that of *M. phaseoli* (Tryon) except in size; length 1·5 mm. Tip of wing beyond distal end of third longitudinal vein; costa reaching M_{1+2}, the section R_{1+5}–M_{1+2} fainter than R_{2+5}–R_{1+5}. Length M-m slightly more (one-and-one-quarter times) than distance from M-m to R-m. Basal veins brown; membrane shining green in

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reflected light; halteres mid-brown. Wing indices*: costal, 3·5 (3·1-3·9); fourth vein, 6·5 (5·8-7·0); 4c, 1·9 (1·8-2·2); 5x, 2·1 (1·8-2·4). Legs concolorous with body, covered with short bristles, pulvilli white. Calypters white with brown fringe. Chaetotaxy: arrangement of thoracic bristles typical of genus; two pairs of strong posterior dorsocentrals† (2+0), the hindmost stronger; no thoracic bristles apart from the postalars lying posterior to the hind pair of dorsocentrals; one strong supra-alar, two postalar (inner weak), one intra-alar (weak, less than half the length of longest dorsocentral), two notopleural, one humeral, one strong

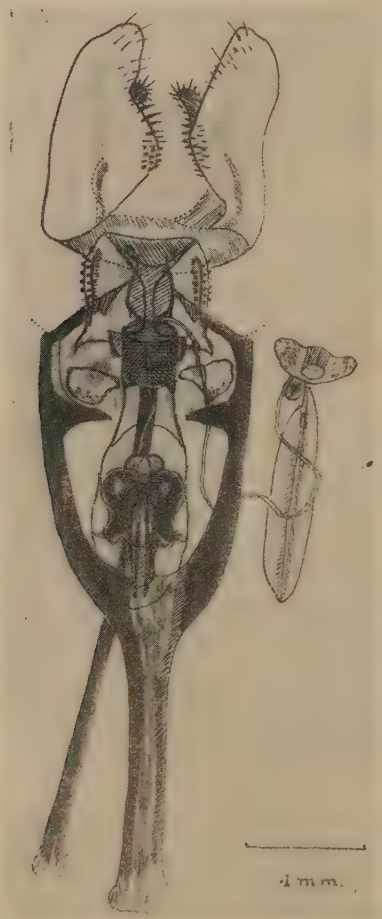

Fig. 1.—Male genitalia of *Melanagromyza wikstroemiae* (ninth tergite out of position).

* As defined by Harrison, R.A. (1959)—Acalypterate Diptera of New Zealand. N.Z. Dep. Sci. Industr. Res., Bull. No. 128.

† Dorsocentral bristles are numbered from the posterior forwards.

Fig. 2.—Leaves of *Wikstroemia indica* showing mines of *Melanagromyza wikstroemiae* with pupae and excretal pattern.

posthumeral (praesutural), numerous short thoracic bristles dorsally, two scutellar (approximately equal in length, the anterior stronger), one propleural, one strong mesopleural, two or three bristles near posterior margin of mesopleuron, that at centre of margin strongest, a group of smaller bristles (usually five or six) in oblique to vertical line near upper centre of sclerite, one strong sternopleural, group of short bristles (usually three or four) below junction of mesopleuron and sternopleuron, vertical line of short bristles on sternopleuron in front of third coxa; one large bristle on each coxa.

Abdomen.—Abdomen covered with short strong bristles. Sixth and terminal segments glossy black in contrast to others which are dull, brown, pruinose. Male and female similar. *Male genitalia* (Fig. 1) (described from paratype slide mounted material), large in comparison with those of *M. phaseoli*. Ninth tergite a large, elongated sclerite bearing bristles on the posterior ventral surface. Surstyli carry numerous short, thick spines and are antero-ventrally situated. Cerci, situated dorsally, relatively small and bristly. Ninth sternite heavily constructed with paired, dorsally directed posterior arms. Hypandrial apodeme atypically long, reaching a short distance anteriorly to the adeagal apodeme; both are long, straight, stout structures in lateral view, reaching well into the third abdominal segment. Pregonites small, bearing ventrally numerous sensory pores (about six) at the anterior extremity. Epiphallus slightly longer than wide. Adeagal hood a well-developed wide structure; phallophore broad, produced

along the adeagal apodeme, the posterior section short and wide. Postgonites barely visible from the ventral side, each as a slender sclerite. Ejaculatory apodeme resembles in form that of *M. phaseoli*. Phallus a short broad structure of the form shown in Fig. 1.

Holotype: Male. From leaf of *Wikstroemia indica* Endl. (Fig. 2). Kenmore, February 1954 (R. P. Kleinschmidt).

Allotype: Female. From leaf of *W. indica*. Coolangatta, January 1959 (A. R. Brimblecombe).

Paratypes: From leaves of *W. indica*. Kenmore, February 1954 (4 specimens and slide mount of male genitalia) (R. P. Kleinschmidt); Coolangatta, January 1959 (15 specimens); and Deception Bay, March 1959 (3 specimens) (A. R. Brimblecombe).

Holotype Reg. No. T5800. Allotype Reg. No. T5801 deposited in Queensland Museum.

MELANAGROMYZA pisi sp. nov.

(Figures 3 and 4)

Length 1·0-1·2 mm (dried specimen); dark brown to black.

Head.—Outer verticals about twice length of postverticals; inner verticals longer; four pairs fronto-orbitals, three superior (dorsally directed), one inferior (inwardly directed), vertical intervals between successive fronto-orbitals increasing from above downwards. Orbital setulae dorsally directed except inferiormost seta which is anteriorly or inwardly directed. Vertical triangle very dark brown to black, pruinose, tapering sharply to centre of frontal vitta. Frontal vitta wide (more than half eye width), dull pruinose. Arista strong, about three times length of visible part of terminal antennal segment, bearing short setae throughout, basal third thickened. Oral vibrissae curved inward, at least twice length of longest seta of suboranial margin. Depth of gena approximately equal to length of oral vibrissa. Occiput dull to pruinose, dark brown, bearing above the occipital foramen a small shining rectangular patch of fine white setae (easily visible at x100 magnification).

Thorax.—Wing venation as for *M. phaseoli*, wings smaller, length 1·3 mm. Tip of wing about half-way between R_{1+5} and M_{1+2}; costa reaching M_{1+2}. Length M-m approximately equal to distance from M-m to R-m. Halteres light to mid-brown. Wing indices: costal, 4·3 (4·0-5·6); fourth vein, 5·9 (5·2-6·8); 4c, 1·4 (1·2-1·8); 5x, 2·8 (2·4-3·0). Legs blackish-brown,

pulvilli paler. Calypters pale grey with dark margin and fringe. Chaetotaxy: thoracic bristles typical of genus: dorsocentrals 2+0 (anterior shorter), postalars only present posterior to hindmost dorsocentrals between which dorsum is bare, one intra-alar (weak, about half length of hind dorsocentral); one supra-alar, two postalar (the inner weak as the dorsal thoracic bristles), two notopleural, one humeral, one posthumeral, two scutellar (anterior three-quarters length of posterior), one propleural, two or three bristles at posterior

Fig. 3.—Male genitalia of *Melanagromyza pisi* (ninth tergite removed).

Fig. 4.—Leaves of *Pisum sativum* showing mines of *Melanagromyza pisi* with pupae and excretal pattern.

edge of mesopleuron, that at centre of sclerite strongest, one strong sternopleural (one shorter anterior to the latter may be present also), group of small bristles at apex or sternopleuron; at least one strong bristle on each coxa.

Abdomen.—Abdomen covered with numerous bristles. Segments posterior to fifth shining black. *Male genitalia* (Fig. 3) (described from allotype and paratype slide mounted material). Ninth tergite a broad, bristly structure. Surstyli directed inwards and not visible in ventral view, with numerous short, sharp spines. Ninth sternite a solid structure with broad side-pieces when viewed ventrally; these carry long posterior arms curving dorsally, and broad antero-ventral processes. Ninth sternite anteriorly produced into a long, broad, hypandrial apodeme lying parallel (when viewed laterally) to the rod-shaped adeagal apodeme, which is a narrower structure reaching further anteriorly than the hypandrial apodeme. Pregonites irregularly shaped plates carried posteriorly to the antero-ventral processes of the ninth sternite, with about four sensory pores at this junction. Epiphallus both long and broad, almost oval viewed ventrally. Adeagal hood, viewed ventrally, hexagonal; phallophore broad. Ejaculatory apodeme a large fan-shaped structure. Phallus produced distally (anteriorly) as two long processes.

Holotype: Female. From leaf of *Pisum sativum* L. (Fig. 4), Toowong, October 1957 (A. R. Brimblecombe).

Allotype: Male. From leaf of *P. sativum,* Toowong, November 1957 (slide specimen of genitalia) (A. R. Brimblecombe).

Paratypes: From leaves of *P. sativum,* Toowong, October 1957 (3 specimens) (A. R. Brimblecombe).

MELANAGROMYZA polyphyta sp. nov.

(Figures 5 and 6)

Length 1·4 mm (dried specimen); dark brown.

Head.—Outer and inner verticals approximately equal in length, the inner slightly longer, about one-quarter their length longer than postverticals; four pairs fronto-orbitals, three superior (dorsally directed), one inferior (inwardly directed), vertical intervals between successive fronto-orbitals decreasing from above downward, the first two intervals approximately equal. Upper superior fronto-orbital longest and strongest. Vertical triangle dark brown to black, dull, tapering below as far as lunule. Frontal vitta wide (more than half eye width), dull black pruinose. Arista about six times length of visible section of

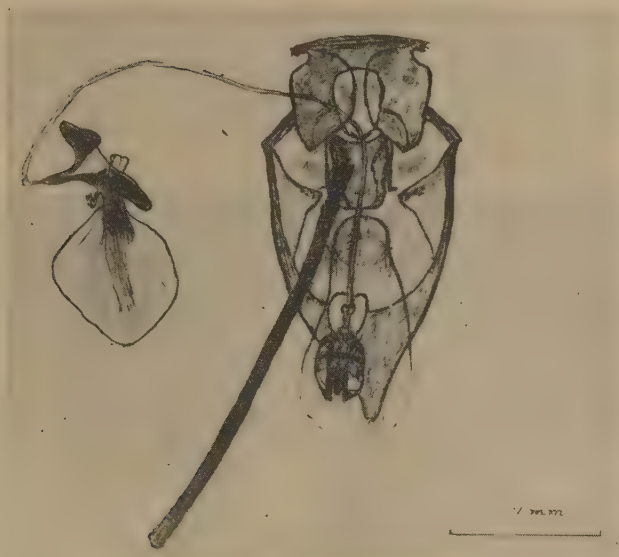

Fig. 5.—Male genitalia of *Melanagromyza polyphyta* (ninth tergite removed).

third antennal segment, bearing short setae throughout; basally thickened. Oral vibrissae about twice length of setae on subcranial margin. Genae narrow; eyes bare. Occipital region dull pruinose, with patch of white hairs long, narrow, centrally placed above occipital foramen (easily visible at x100 magnification).

Thorax.—Mean wing length 1·4 mm. but widely variable. Tip of wing halfway between distal ends of R_{1+5} and M_{1+2}; costa reaching M_{1+2} but section R_{1+5}–M_{1+2} scarcely strengthened. Length M–m greater (one-and-a-third times) than distance from M–m to R–m. Halteres dark brown. Wing indices: costal, 4·2 (3·7–5·1); fourth vein, 8·0 (6·4–9·0; 4c, + 2·2 (1·7–2·7); 5x, 2·0 (1·8–2·6). Legs concolorous with body, pulvilli pale. Calypters grey with black edge and fringe. Chaetotaxy: dorsocentrals 2+0, posterior longer; one supra-alar, two postalar (inner short), one intra-alar (fine, short, longer than inner postalar), two notopleural, one humeral (with numerous short bristles anterior to it), one posthumeral, two scutellar (anterior longer), one propleural, one mesopleural (numerous short bristles surrounding its base), two sternopleural at apex (apical longer), a group of short bristles on the anterior edge of the sternopleuron, several bristles below junction of mesopleuron and sternopleuron, including one long bristle posteriormost; several bristles on coxae.

Abdomen.—Male genitalia (Fig. 5) (described from paratype slide mounted material). Ninth sternite viewed ventrally with narrow sidepieces bearing dorsally curved posterior arms and very broad antero-ventral processes;

anteriorly produced into a short, blunt hypandrial apodeme. Adeagal apodeme a long, slender rod reaching considerably further anteriorly, to the anterior edge of the fourth sternite. Pregonites not visible in preparations as separate structures, but several pores present at the posterior edges of the antero-ventral processes; (these may constitute a modification of the two combined structures).

Fig. 6.—Leaves of *Passiflora suberosa* showing mines and pupae of *Melanagromyza polyphyta*.

Surstyli broad, sclerotized structures with several short, sharp spines, scattered over their surface and long bristles on the inner edge. Epiphallus oval, widest posteriorly. Adeagal hood a wide structure, anterior to which is the broad phallophore. Ejaculatory apodeme a large, roughly fan-shaped structure with a broad, angular base. Phallus short compared with other species of the genus.

Holotype: Female. From leaf of *Passiflora suberosa* L. (Fig. 6), Kenmore, February 1957 (R. P. Kleinschmidt).

Allotype: Male. Data as for holotype.

Paratypes: From *P. suberosa* as for holotype (1 specimen); Kenmore, April 1953 (3 specimens) (R. P. Kleinschmidt); Kenmore, February 1956 (slide specimen of male genitalia) (R. P. Kleinschmidt); Kenmore, February 1959 (2 specimens) (R. P. Kleinschmidt).

Holotype Reg. No. T5802, Allotype Reg. No. T5803 deposited in Queensland Museum.

MELANAGROMYZA indigoferae sp. nov.

(Figure 7)

Length 1·0 mm (dried specimen); dark brown.

Head.—Outer verticals shorter than inner verticals, equal in length to postverticals; four pairs fronto-orbitals, three superior (dorsally directed), one inferior (inwardly directed), first and second bristles longer and coarser. Vertical triangle concolorous with head, tapering to a point at lunule. Frontal vitta almost as wide as eye. Antennae divergent distally, contiguous basally. Arista about twice length of segment from which it arises, bearing fine setae and basally thickened. Oral vibrissae longer than depth of genae, about one-sixth eye height. Occipital region dull pruinose, with no distinct pattern visible on specimens examined although setae present.

Thorax.—Wing venation dark, especially at wing base. Wings with metallic lustre, length 1·2 mm. Costa reaching M_{1+2} the last section (R_{4+5}–M_{1+2}) scarcely thickened; length of M-m slightly less than distance from M-m to R-m. Halteres brown. Wing indices: costal, 3·4 (3·0–3·6); fourth vein, 6·6 (6·2–7·2); 4c, 2·0 (1·9–2·0); 5x, 2·6 (2·1–3·2). Legs concolorous with body. Calypters grey with black edge and fringe. Chaetotaxy: dorsocentrals 2+0 (posterior shorter), one supra-alar, two postalar (the inner short and

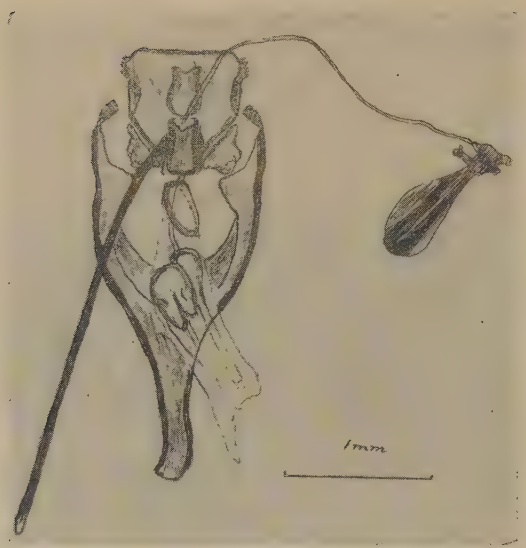

Fig. 7.—Male genitalia of *Melanagromyza indigoferae* (ninth tergite removed).

resembling the short thoracic bristles, one intra-alar (very short, not easily distinguishable from short thoracic bristles), two notopleural (not as strong as some of the thoracic bristles, e.g. humeral, posthumeral), one humeral (anterior to which numerous small bristles are present), one posthumeral, two scutellar (posterior more than twice length of anterior), one propleural, one mesopleural (centrally placed on posterior edge of sclerite; other bristles may be present on this sclerite, near the mesopleural bristle or at the dorsal edge), two sternopleural (posterior longest, situated just beneath junction with mesopleuron; at least one long bristle on the coxa.

Abdomen.—Male genitalia (Fig. 7) (described from paratype slide mounted material). Ninth sternite broad, with side-pieces extended into wide anteroventral processes and short posterior arms. Hypandrial apodeme long, broad and curved ventrally at distal end. Adeagal apodeme long, narrow, rodlike, reaching anteriorly beyond hypandrial apodeme. Phallophore long, slender at posterior end.

This species is smaller than most others in the genus and the male genitalia are correspondingly small. In general the form of the genitalia resembles that of *M. pisi*.

Holotype: Male: From leaves of *Indigofera suffruticosa* Mill., Eight Mile Plains, May 1955 (R. C. Colbran).

Allotype: Female. Data as for holotype.

Paratypes: From *I. suffruticosa* as for holotype (3 specimens and two slides of male genitalia).

Holotype Reg. No. T5806, Allotype Reg. No. T5807 deposited in Queensland Museum.

MELANAGROMYZA dianellae sp. nov.

(Figures 8 and 9)

Length 1·5 mm (dried specimen); shining black, calypters shining white with pale brown margin and white fringe.

Head.—Outer verticals about twice length of postverticals and stronger, approximately same length as inner verticals; four pairs fronto-orbitals (three superior, one inferior), vertical intervals between successive fronto-orbitals increasing from above downwards; orbital setulae directed dorsally except lowermost which is directed forwards. Vertical triangle tapering to centre of frontal vitta opposite second pair of fronto-orbitals. Frontal vitta wide (more than half eye width), dull pruinose. Antennae close together at base, arista basally thickened, covered with very short setae for entire length. Genae narrow, about equal in depth to half length of oral vibrissae, margin rounded. Occipital setae forming a V (visible at x100 magnification) of width subequal to the distance between the hindmost ocelli.

Thorax.—Wing length 1·4 mm. Costa reaching M_{1+2} strongly; length of M-m only slightly greater than distance from M-m to R-m. Halteres mid-brown. Wing indices: costal, 3·9; fourth vein, 6·5; 4c, 1·75; 5x, 3·0. Legs black. Chaetotaxy: dorsocentrals 2+0 (anterior slightly more than half length of posterior), one supra-alar, two postalar (inner weak), one intra-alar, two notopleurals, one humeral, one posthumeral, two scutellar (hind pair longer and stronger), one propleural, one mesopleural (situated centrally on hind margin of sclerite, with small bristles on hind margin also), one strong sternopleural (a weaker bristle is usually present anterior to this), postalars almost in line with hindmost dorsocentrals but may be slightly posterior. The bristles are not noticeably thickened at the base.

Abdomen.—Male genitalia (Fig. 8) (described from paratype slide mounted material). Ninth sternite well developed. Hypandrial apodeme very long and broad, reaching as far forward as the adeagal apodeme. Surstyli not visible from ventral view. Pregonites broad, bearing about six pores anteriorly. Epiphallus long and moderately narrow. Adeagal hood broad, long and rectangular; phallophore very short. Ejaculatory apodeme shaped similarly to an isosceles triangle, the bulb small. Phallus bearing two very long processes distally.

Holotype: Female. From leaf of *Dianella caerulea* Sims (Fig. 9), Coolangatta, April 1956 (A. R. Brimblecombe).

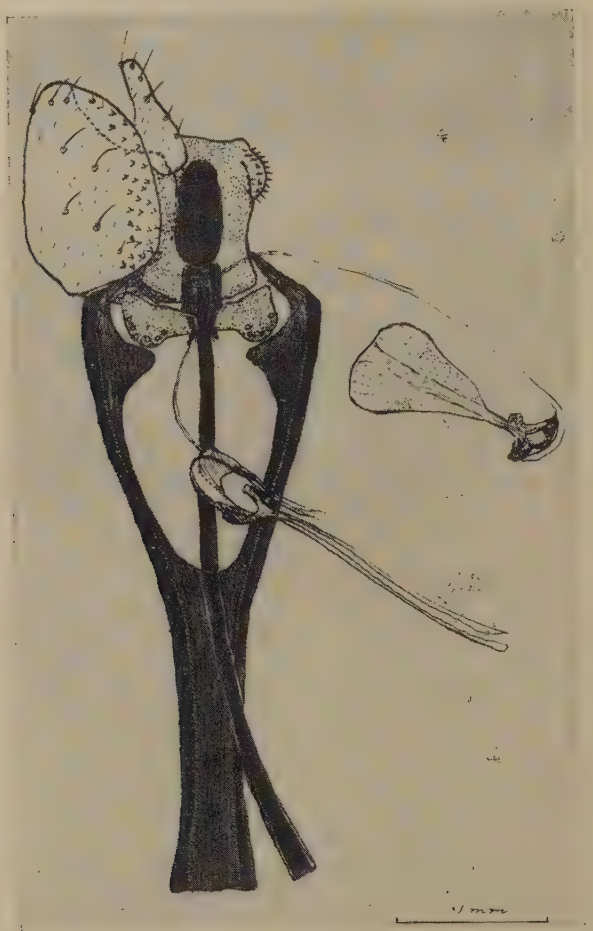

Fig. 8.—Male genitalia (ventral view) of *Melanagromyza dianellae* (with half ninth tergite removed).

Fig. 9.—Leaves of *Dianella caerulea* showing mines, pupae and excretal pattern of *Melanagromyza dianellae*.

Allotype: Male. From leaf of *D. caerulea,* Coolangatta, June 1960 (A. R. Brimblecombe).

Paratypes: From leaves of *D. caerulea,* Coolangatta, April 1956 (two specimens); January 1959 (slide specimen of male genitalia); June 1960 (seven specimens) (A. R. Brimblecombe).

Holotype Reg. No. T5808, Allotype Reg. No. T5809 deposited in Queensland Museum.

HAPLOMYZA caulophaga sp. nov.

(Figure 10)

The species agrees closely with *H. imitans* Malloch in external characters, with the following exceptions:

Abdominal segments widely clear yellow on hind margins, rest of segments black, disc of thorax mid-grey; four fronto-orbitals (occasionally five); orbital setulae situated below level of superior fronto-orbitals; four pairs dorsocentrals, the front two pairs weaker but still strong; fourth vein reaching a point at central margin of wing-tip but in front of the widest part of the wing.

Head.—Head yellow, ocellar triangle and occiput black, aristae, bristles and hairs black. Four fronto-orbitals (five in one male specimen), upper two superior, lower two inferior, orbital setulae very few and situated near inferior fronto-orbitals.

Thorax.—Thorax greyish dorsally, not glossy, yellow laterally, scutellum yellow with lateral anterior corner black, extending to include hindmost pair bristles; mesopleuron with black spot on lower anterior angle, sternopleuron black except at upper margin, squamae whitish with pale fringe. Dorsocentrals 3+1, the front two weaker than the hind two, but well-developed, the third pair almost in line with the suture, the intradorsocentral hairs continuing forward from a position slightly posterior to the second pair of dorsocentrals. Small hairs in dorsocentral line in front of the bristles. Fourth vein ending exactly at centre of wing-tip but wing reaching slightly further laterally behind distal end of this vein.

Abdomen.—Ovipositor shining black with last abdominal segment chiefly yellow; abdomen brownish-black except for hind margins of tergites, which are clear yellow. *Male genitalia* (Fig. 10) (described from allotype). Ninth tergite very broad, bearing one short tooth at anteroventral angle; near this the surstylus on each side is separated from the tergite by a suture; surstyli bearing about six strong bristles; cerci short and fairly broad, carrying numerous bristles. Ninth sternite long, V-shaped, anterior end rounded, posterior end widened, very little dorso-ventral curvature, side-pieces posteriorly thickened and curved first forwards and then backwards. Pregonites united to this process, approximated at the centre. Adeagal apodeme long, reaching posteriorly to the anterior margin of the ninth tergite. Epiphallus concave anteriorly. Adeagal hood very broad, bearing ventrally two short processes; phallophore long. Postgonites long and irregularly shaped. Distal end of phallus brush-like.

Holotype: Female. From stalk of silver-beet (*Beta vulgaris* L. var. *cicla* L.) Nudgee, October 1951 (R. P. Kleinschmidt).

Allotype: Male. Data as for holotype (slide specimen of genitalia).

Holotype Reg. No. T5810, Allotype Reg. No. T5811 deposited in Queensland Museum.

Fig. 10.—Male genitalia of *Haplomyza caulophaga.*

MELANAGROMYZA phaseoli (Tryon).

Synonymy:

Oscinis phaseoli Tryon, 1895.

Oscinis fabae Tryon, 1897 (Nomen nudum).

Agromyza phaseoli Coquillett, 1899.

Agromyza (*Melanagromyza*) *phaseoli* Coquillett; Malloch, 1927.

Melanagromyza phaseoli (Coquillett); Greenwood, 1929.

Coquillett has formerly been credited as the authority for this species.

Although Tryon stated that the name *Oscinis phaseoli* was provisional, the description provided is considered adequate. An extract from his article "The Bean Maggot," dealing with the descriptive aspect is given below.

"The living insects . . . are small, black, two-winged flies which are provisionally named *Oscinis phaseoli* . . . The maggot is a translucent, yellowish, elongate, cylindrical, twelve-jointed grub, obtuse at each extremity, and rather broad towards the head; it measures 1¼ to 1½ lines in length . . . The puparium is a parallel-sided object rounded at each end and about 1 line in length . . . The fly, which is quite a small though conspicuous insect, with its wings directed obliquely backwards, is black, but its eyes present beautiful copper-coloured and green reflections, and its wings have an opalescent glimmer when viewed in certain positions . . . The pest is assisted in its progress through the plant-tissue by a large and stout hook-shaped toothed apparatus in front of the mouth, and by rows of sharp papillae just behind the head, and at the joints separating each body segment."

KEY TO SPECIES

Seven species in the genus *Melanagromyza* are known from Queensland. These may be determined as follows.

Key to Queensland Species of MELANAGROMYZA

1. Fronto-orbitals 2 + 2 2
   Fronto-orbitals 3 + 1 3

2 (1) Calypters white ..... *albisquama* (Malloch)
   Calypters grey ..... *phaseoli*

3 (1) Anterior scutellar bristles shorter than posterior 4
   Anterior scutellar bristles longer than posterior ..... *polyphyta*
   Anterior scutellar bristles approximately equal in length to posterior ..... *wikstroemiae*

4. (3) Body black, calypters white ..... *dianellae*
   Body dark brown; calypters grey 5

5. (4) Anterior scutellar ½ or less than ½ length posterior scutellar bristles ..... *indigoferae*
   Anterior scutellar ¾ length posterior scutellar bristles ..... *pisi*

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DROUGHT FEEDING STUDIES WITH CATTLE

5. The Use of Sorghum Grain as a Drought Fodder for Non-pregnant Heifers

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SUMMARY

Four groups of 10 maiden Hereford heifers, approximately 16 months of age, were group-fed in bare yards for 26 weeks. All groups received crushed sorghum grain, supplemented with 1 per cent. ground limestone, at the rate of 3 lb per head per day. Daily feeding with and without a supplement of 0.5 per cent. sodium chloride added to the grain was compared with twice-weekly and weekly feeding without added salt.

Two heifers died during the feeding period.

At the commencement of all-grain feeding the heifers averaged 462 lb body-weight. During the period of grain feeding the animals lost weight rapidly during the initial stages, but thereafter the rate of loss declined. After 26 weeks heifers fed daily lost an average of 105 lb, those fed twice-weekly 98 lb, and those fed weekly 129 lb. Although animals receiving 0.5 per cent. sodium chloride in the grain lost an average of only 83 lb, subcutaneous oedema of the submandibular space occurred in six animals in this group. All groups rapidly regained their lost weight when returned to pasture.

The incidence of geophagia was high in all groups, appearing to be more common in the daily-fed groups. Rumination ceased within two weeks of all-grain feeding, but resumed in all animals within 48 hours of either being returned to pasture or fed lucerne chaff. At the conclusion of grain feeding, animals from the two intermittently fed groups had a better general appearance and were more active than animals fed daily.

After 26 weeks of all-grain feeding the haemoglobin, packed cell volume and red cell count of animals in the two intermittently-fed groups were comparable with initial values, whereas these values were significantly lower than initial values in animals from the groups fed daily. In a comparison of the two groups of intermittently-fed animals, those fed twice-weekly had a significantly higher haemoglobin concentration, packed cell volume and red cell count. Animals from this group also had higher blood and plasma volumes than those from the other three groups.

There was a decline in serum protein level due largely to a decrease of serum albumin. Lowest levels were recorded in animals fed 0.5 per cent. sodium chloride daily, but the results did not indicate that oedema was the result of a deficiency in circulating protein.

Although there were statistically significant differences at different sampling periods within groups, and between groups at 26 weeks in levels for prothrombin time; blood inorganic phosphate; serum calcium and magnesium; plasma chloride, sodium and potassium, these levels did not indicate a pathological change at any sampling period.

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The mean liver vitamin A reserves declined in all groups, but there was no clinical evidence of night blindness in heifers after 26 weeks on a ration containing negligible carotenoids.

Under the conditions of this experiment, twice-weekly feeding of restricted sorghum grain for survival appeared more satisfactory than either daily or weekly feeding. The inclusion of sodium chloride with the grain was not beneficial.

I. INTRODUCTION

In introducing this series of papers, Morris (1958*a*) made reference to grains as one of the classes of feedstuffs available in Queensland for the drought feeding of cattle. It was considered that of the grains, sorghum grain probably had the greatest potential for production and storage on beef cattle properties. In another paper of the series, Morris (1958*b*) reported a preliminary experiment in which eight beef-type maiden heifers were individually fed 3 lb of crushed sorghum grain per head per day for a period of 27 weeks.

The drought feeding experiment reported in this paper was designed to study the effects on beef cattle of restricted feeding of crushed sorghum grain under group feeding conditions, to compare daily with intermittent feeding, and to evaluate supplementation of the grain with 0·5 per cent. sodium chloride. Data were obtained on changes in body-weight and body measurements, feed consumption time, and animal behaviour. Determinations were made on prothrombin time; blood haemoglobin, packed cell volume, red cell count, and inorganic phosphate; serum calcium, magnesium, total protein, albumin and globulin; plasma chloride, sodium and potassium; liver vitamin A; plasma volume and extracellular fluid volume.

II. MATERIALS

Experimental Yards and Facilities.—The experiment was conducted in four adjacent open yards (each 40 ft x 48 ft) of identical design. The yards and an area of approximately 2 ft around the perimeter of the unit were maintained bare of vegetation by mechanical means.

Each yard was provided with a feed trough, 28 ft in length, protected from the weather by a hip roof 12 ft wide. Water was available *ad lib.*, one water trough in the dividing fence serving two yards.

Experimental Animals.—Forty-eight dehorned maiden Hereford heifers, approximately 16 months of age and in store condition, were selected on a beef cattle property in the Burnett district of Queensland. The selection was made on the basis of uniformity of body-weight and conformation. They were transported to the Animal Husbandry Research Farm, Rocklea, Brisbane. During the next four weeks these animals grazed predominantly *Paspalum dilatatum* pastures.

All animals were tested and inoculated as described by Morris (1958*a*) and drenched with phenothiazine. All gave negative results to the single intradermal test for tuberculosis and to serological tests for brucellosis, contagious bovine pleuropneumonia, *Leptospira pomona* and *L. hyos.*

Sorghum Grain.—A single consignment of *Sorghum vulgare* L. grain (Alpha variety) was purchased at the commencement of the experiment. It was stored in bags in a closed shed during the experimental period. The grain was crushed in two portions, each of approximately 5 tons. The first portion was crushed immediately prior to the commencement of the experiment, the remaining 5 tons when the first portion had been almost consumed.

Water.—The source of water for the cattle was the Brisbane City supply.

III. METHODS

Body-weight.—Individual animals were weighed on a cattle weighing scale with an accuracy of ± 2 lb.

Body Measurements.—The height of the withers at their highest point was measured by the technique illustrated by Brody (1945). The heart girth was measured by the technique used by Brody (1945), with the modification that a constant tension of 7 lb was exerted on the steel tape by a spring balance. All readings were done in triplicate.

Sampling and Analysis of Grain.—A seed trier was used to obtain grain samples from both crushings. After each crushing, samples from three sites in each bag were taken from a random selection of approximately one-fifth of the bags and bulked for analysis. Proximate analysis of feed was determined by the method described by the Association of Official Agricultural Chemists (1955). Grain particle size was determined by using a Greebrun laboratory sieve.

Sampling and Analysis of Water.—Equal aliquots of water were obtained at weekly intervals from the point of supply and bulked in a polythene container for analysis. Chloride was determined by electrometric titration against a silver nitrate solution. Sodium and potassium were determined using an Eel flame photometer.

Examination of Faecal Samples.—The method for worm egg counts was that described by Roberts and O'Sullivan (1950).

Meterological Data.—Daily rainfall and maximum and minimum tempera- were recorded at a site approximately 500 ft from the experimental yards.

Prothrombin Time.—Prothrombin time was determined, using oxalated blood, by the technique of Quick (1938).*

Haemoglobin.—Heparinized blood was used for the determination of haemoglobin by the method of Donaldson *et al.* (1951).

Packed Cell Volume (P.C.V.).—Essentially the method of Wintrobe (1947) was used, employing heparinized blood and a relative centrifugal force of approximately 2067 *g*.

Red Cell Count (R.C.C.).—Heparinized blood was taken for R.C.C. The bulk dilution technique (Dacie 1956), using Vallerino's diluting solution, was followed.

Blood Inorganic Phosphate.—The method of Moir (1945) was used for this determination.

Serum Calcium.—Serum calcium was determined by a modification of the method of Clark and Collip (1925).

Serum Magnesium.—Serum magnesium was determined by a modification of the method of Holzapfel (1934).

Serum Total Protein, Albumin and Globulin.—Serum proteins were determined by the method of Gornall, Bardawill and David (1949).

Plasma Chloride, Sodium and Potassium.—Samples were obtained and analysed for plasma electrolytes as described by Morris (1958*b*).

Liver Vitamin A.—Liver samples were obtained by the aspiration biopsy technique of Loosemore and Allcroft (1951), using the instruments described by Dick (1952). Liver cores were expelled into 2 per cent. aqueous hydroquinone and washed free from blood and adhering tissue. Extraneous fluid was removed on filter paper and the core transferred to stoppered, tared flasks containing 12·5 ml of 1 per cent. alcoholic potassium hydroxide. The flasks were re-weighed and 0·4 ml of 10 per cent. hydroquinone in alcohol (96 per cent.) was added as an antioxidant. The contents were then saponified and in the course of saponification the cores were broken up, using a glass rod. The extraction and determination of liver vitamin A was as described by Gartner and Ryley (1956). Extraction was commenced approximately 90 min after the samples were obtained.

Plasma Volume, Blood Volume and Extracellular Fluid Volume (E.C.F.V.).—A sample blank of heparinized blood was withdrawn from the jugular vein. Through the same needle 20 ml of 0·5 per cent. Evans Blue

* Thromboplastin was prepared from desiccated rabbit brains by the Commonwealth Serum Laboratories, Melbourne.

(T-1824) solution was injected, followed by 50 ml of 10 per cent. sodium thiocyanate solution. At 20 min after injection a sample was taken from the opposite jugular vein and Evans Blue was determined in plasma by the method of Gregersen (1944). At approximately two, three and four hours after injection, samples were taken and thiocyanate estimated by the method of Bowler (1944). In the computation of E.C.F.V. a correction was made on the basis of a 7 per cent. protein content in plasma. All measurements were made using a Hilger spectrophotometer. Blood volume was calculated as the product of

$$\frac{\text{plasma volume} \times 100.}{\text{P.C.V.}}$$

IV. EXPERIMENTAL

(a) Design

Forty heifers were retained after elimination of eight animals, which were extremes in body-weight. These were arranged in descending order of weight and formed into 10 blocks (1-10), each block consisting of four animals of comparable weight. One animal in each block was allotted at random to each of four groups.

The animals were removed from pasture, transferred to yards in their respective groups, and changed to the all-grain ration over a period of three weeks according to the following schedule:—

Days	Ration (lb/head/day) Fed Daily	
	Sorghum Grain	Lucerne Chaff
1–4	2·0	4·0
5–8	2·5	3·0
9–12	2·5	2·0
13–17	2·75	1·25
18–21	3·0	0·5

At the conclusion of this period, the groups were fed their experimental rations. The group and experimental treatments were as follows:—

Group	Ration (3 lb/head/day)	Frequency of Feeding
I	Sorghum grain + 1 per cent. limestone	Daily
II	Sorghum grain + 1 per cent. limestone + 0·5 per cent. sodium chloride	Daily
III	Sorghum grain + 1 per cent. limestone	Twice weekly
IV	Sorghum grain + 1 per cent. limestone	Weekly

The feed was distributed evenly along the 28 ft of troughing at each feeding. Animals were fed between 9 a.m. and 10 a.m. Group III received 9 lb per head each Monday morning and 12 lb per head each Thursday morning, while Group IV received 21 lb per head each Monday morning.

The all-grain feeding continued for 26 weeks. At the conclusion of this period, five animals from each Group (Blocks 1, 2, 6, 9 and 10) were turned out on predominantly *Paspalum dilatatum* pastures. The remainder were retained in the yards and fed lucerne chaff *ad lib.* for one week, in order to determine their average daily intakes of dry matter. They were then turned out on the same pasture. Observations on these animals continued for a further eight weeks.

(b) Observations and Recordings

Body-weights were obtained at weekly intervals throughout the whole period. Weighings were made between 7 a.m. and 8 a.m. each Monday morning. Water was available prior to weighing, but the animals had not been fed.

Body measurements were made during the last week on pasture prior to grain feeding and on the last day of the grain-feeding period.

Prothrombin time; blood haemoglobin, P.C.V., R.C.C., inorganic phosphate; serum calcium, magnesium, total protein, albumin and globulin; and plasma chloride, sodium and potassium were determined on samples from all animals on three occasions during the experiment. Initial determinations were made during the last week the animals were grazing, i.e. three weeks before they were on all-grain rations. Subsequent analyses were made at 12 and 26 weeks of all-grain feeding. Further determinations of blood haemoglobin and P.C.V. were done on all animals after six weeks on pasture following the period of all-grain feeding.

Initial determinations of plasma volume, blood volume, E.C.F.V. and liver vitamin A were made on five animals in each group during the last week on pasture and repeated at 26 weeks of all-grain feeding. The animals used were from Blocks 1, 2, 6, 9 and 10. One of these animals in Group I died and for the final determination was replaced by the animal from the same group that was closest to it in weight at the beginning of the experiment.

The animals were observed feeding on five days each week to obtain grain consumption times and to observe animal behaviour. They were observed for three other periods, of approximately 15 min each, during the daylight hours of these five days for evidence of geophagia, wood-chewing, coat-licking and rumination, and to obtain grain consumption times for Group IV.

Faecal samples were obtained at intervals of four weeks from five animals selected at random from each group. Egg counts were made to indicate whether further anthelmintic treatment against internal parasitism was necessary.

V. RESULTS

(a) Analyses of Grain and Water

The proximate analysis of the grain expressed in percentages was as follows:—

Moisture	Protein	Fat	Fibre	N.F.E.	Ash	Ca	P	Cl	Na	K
12·2 ..	12·3	1·3	2·2	70·7	1·3	0·04	0·24	<0·001	0·02	0·38

Examination for particle size of the crushed grain showed that 86 per cent. of the particles were between 0·24 and 0·10 cm in diameter.

The composite water sample obtained during the experimental period had the following concentration of ions in m-equiv./l: chloride 2·45, sodium 1·45 and potassium 0·05.

(b) Mortality

Two heifers died during the period of grain feeding. One, initially the lightest in Group I, died during the 12th week. It became progressively weaker and at the time of death had lost 80 lb in body-weight compared with the group mean loss of 68 lb. The animal was recumbent on the day prior to death and bloating became marked. The significant autopsy findings were emaciation and distension of the rumen with an estimated 8-10 gal of gruel-like liquid. Little fat was present in the carcass.

One animal in Group III died overnight during the 16th week. It had not shown any marked symptoms and its weight at the previous weighing was slightly higher than the mean of the group. It was in poor condition, but at autopsy fat was still present in the subcutis and abdominal depots. The rumen contents weighed 46 lb. They were very fluid and contained grain, soil and approximately 70 hair-balls varying from 1 to 5 cm in diameter. One hair-ball was present in the abomasum and another in the duodenum. There was little ingesta distal to the site of the hair-ball in the duodenum, but the hair-ball was freely movable and appeared unlikely to have caused complete obstruction. Ash analyses indicated that approximately 30 per cent. of the dry-matter content of the rumen was soil.

(c) Body-weight

The group mean body-weight determined at weekly intervals during the periods of grain feeding and recovery is shown in Figure 1. The group mean body-weight at five periods—at the commencement of yard feeding, at the commencement of all-grain feeding, after 12 and 26 weeks of all-grain feeding, and after a recovery period of 9 weeks on pasture—is shown in Table 1. During the initial stages of grain feeding animals lost body-weight rapidly, but thereafter the rate of loss declined. After 12 weeks and also at the end of grain feeding, Group IV had lost significantly more weight than Group II ($P < 0{\cdot}001$) and Group III ($P < 0{\cdot}005$). No other weight differences were significant.

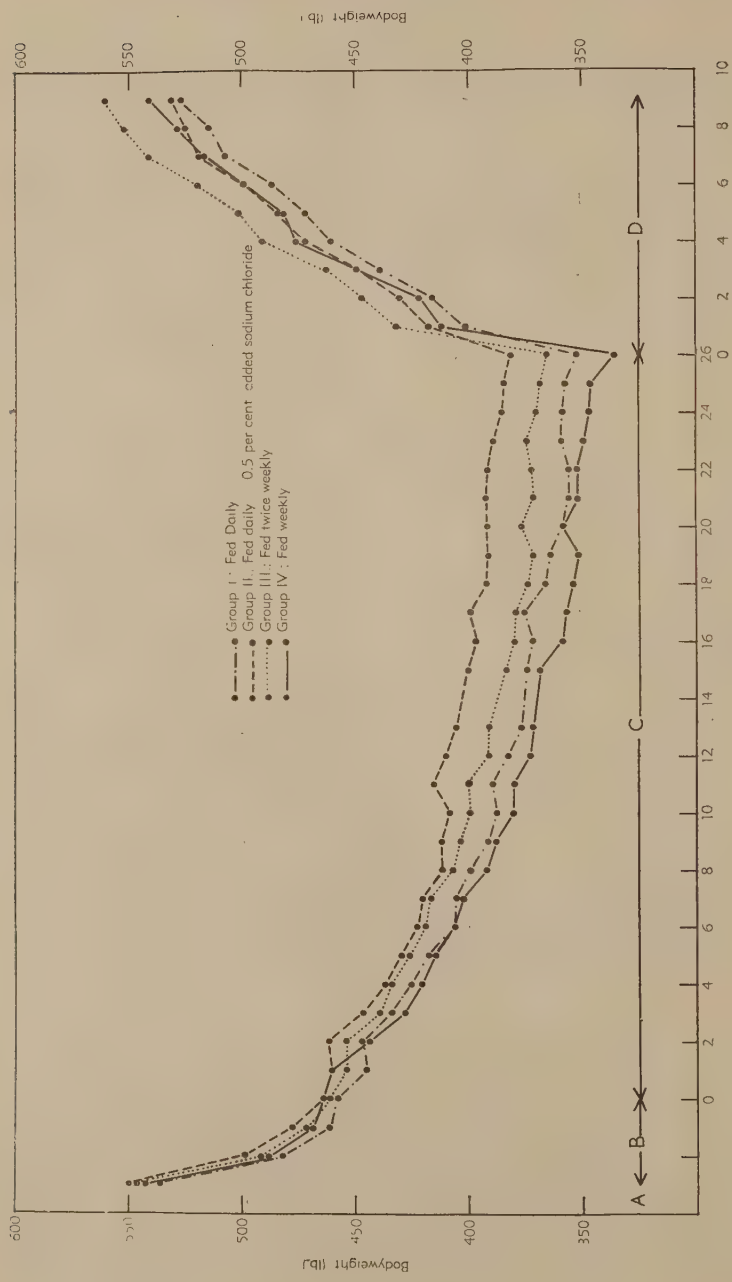

Fig. 1.—Weekly body-weights of heifers prior to, during and after feeding 3 lb crushed sorghum grain per head per day. A, pasture. B, grain/lucerne chaff mixture. C, all-grain. D, recovery on pasture.

TABLE 1

Group Mean Body-weight of Heifers Fed 3 lb Crushed Sorghum Grain per Head per Day

Date	Period	Group Mean Body-weight (lb)			
		I	II	III	IV
19–v–58	Commencement of yard feeding (from pasture)	536	550	547	542
9–vi–58	Commencement of all-grain feeding	457	464	462	464
1–ix–58	After 12 weeks of all-grain feeding	384	409	391	373
8–xii–58	After 26 weeks of all-grain feeding	352	381	364	335
10–ii–59	After 9 weeks' recovery on pasture	520	530	560	540

During the first week of the recovery period the animals fed lucerne chaff showed no statistical differences in body-weight compared with the animals on pasture. Therefore all animals have been used for the assessment of weight gain after grain feeding. During the period of 9 weeks Group IV gained more weight than Group I ($P < 0{\cdot}001$) and Group II ($P < 0{\cdot}01$); Group III gained more weight than Group I ($P < 0{\cdot}05$) and Group II ($P < 0{\cdot}001$). The rapid loss in weight during the introduction to all-grain feeding and the rapid gain during the first week of recovery on pasture are partly due to changes in gastro-intestinal fill.

There were no significant differences in body-weight between groups when initial weights obtained on pasture just prior to the commencement of yard feeding (May 19, 1958) were compared with those obtained after 9 weeks' recovery from grain feeding (February 10, 1959).

(d) Body Measurement

The group means of the height at withers (height) and heart girth of heifers at the time of confinement to yards and also at the conclusion of grain feeding are shown in Table 2. The height increased slightly in all groups, the increase being significant ($P < 0{\cdot}01$) in Groups III and IV. The heart girth decreased significantly ($P < 0{\cdot}001$) in all groups. There was no significant difference between groups in any of these measurements.

TABLE 2

Body Measurements of Heifers Fed 3 lb Crushed Sorghum Grain per Head per Day

Group	Height (cm)		Heart Girth (cm)	
	Initial*	Final†	Initial*	Final†
I	104·5	105·4	142·7	133·2
II	105·9	106·1	142·1	135·2
III	104·5	106·5	141·6	133·2
IV	105·2	106·8	142·2	131·2

*Initial measurements were made during the last week on pasture, prior to grain feeding.

†Final measurements were made on the last day of the 26 weeks' grain-feeding period.

(e) Grain Consumption Time

Animals in Groups I, II and III remained at the trough until all their food was consumed. The average grain consumption times for these groups were as follows:—Group I, 17 min; Group II, 15 min; Group III (Mondays, 9 lb), 53 min; and Group III (Thursdays, 12 lb), 74 min. The rate of grain consumption of these groups was therefore similar, being 1 lb per 5-6 min. There was little change in the rate of consumption as the experiment progressed.

The time required for Group IV to consume their weekly feed was variable. When presented with their first weekly feed, the animals consumed more than 99 per cent. of their ration within 5·3 hr. Thereafter, it required between 48 and 100 hr (average 65 hr) for the animals to consume all their weekly feed.

(f) Animal Behaviour

Geophagia commenced in some animals during the second week of all-grain feeding. There was an increasing incidence of earth-eating until the 12th week, after which it remained relatively constant. During the whole period of grain feeding, earth-eating appeared to be more common in the daily-fed groups. The incidence relative to Group I based on the number of animals observed eating earth during the daily observation periods was as follows:—Group II, 119 per cent.; Group III, 46 per cent.; and Group IV, 55 per cent.

Attempts to chew the wood on the troughs and the rails of the yards were noted during the first few weeks of grain feeding, but thereafter they were seen infrequently. Animals in all groups were frequently observed to lick the coats of other animals in the group.

No animal was observed ruminating after two weeks on all-grain feeding, but "pseudo-rumination" (chewing without a bolus being regurgitated) was observed occasionally during the remainder of the period. After the conclusion of grain feeding, one animal was observed to begin ruminating within 30 min of eating lucerne chaff, and all animals, whether fed lucerne chaff or grazing, were seen ruminating within 48 hr.

The observations made at feeding indicated that, although there was a sporadic incidence of heifers being prevented by others from obtaining a fair share of the ration, no one animal was consistently in this category. One animal in Group I, which died during the 12th week of grain feeding, was tardy in approaching the trough at feeding and did not always remain at the trough until all feed was consumed.

Animals in Group IV were lethargic after they had consumed their first weekly feed. They showed inappetence at the next feed, and one animal was not observed to eat for two weeks after the initial weekly feed.

As the experiment progressed, the animals in all groups became more dejected and lethargic. At the termination of all-grain feeding, Groups III and IV were noticeably more active than Groups I and II. The coats of animals in Groups III and IV did not appear to contain as much debris and soil as those in Groups I and II. At the conclusion of grain feeding, Group III, as assessed by a number of independent observers, had a better general appearance than Group IV, which in turn was better than the other two groups.

During the last few weeks of grain feeding, some of the animals in Group II developed slight subcutaneous oedema of the submandibular space. At the conclusion of grain feeding, six animals in Group II were affected. Oedema was not apparent in any animals in the other groups.

(g) Examination of Faecal Samples

The faeces of the animals in all groups varied in consistency from very firm to semi-liquid. As voided, they always contained visible particles of grain, and had an odour similar to faeces from pigs fed on rations containing a high proportion of cereal grains.

Faecal samples obtained from a random sample of five animals per group at intervals of four weeks showed that 95 per cent. of the samples had less than 100 eggs per gram (e.p.g.), and all except one had less than 200 e.p.g. This sample had 420 e.p.g. All eggs were of *Cooperia* spp. These results, particularly when considered in relation to the low faecal output of these animals, would indicate that internal parasites were not a complicating factor in the experiment.

(h) Feed Intake During the Week after Cessation of Grain Feeding

The *ad lib.* daily mean lucerne chaff consumption, on a dry-matter basis, during the week after cessation of grain feeding is shown in Table 3. During the first few days, the daily dry-matter consumption of Groups I and II was similar to their previous daily intake of grain, and much less than that of Groups III and IV. The daily intakes of the two former groups gradually increased during the week, and at the end of the week approached those of Groups III and IV.

TABLE 3

Mean Daily Consumption of Lucerne Chaff after Cessation of the Grain-feeding Period

Day	Mean Dry-matter Consumption per Head per Day (lb)			
	Group I	Group II	Group III	Group IV
1	3·4	4·8	7·2	7·4
2	2·5	2·4	8·0	6·4
3	3·8	3·7	6·2	7·0
4	5·3	4·5	7·6	6·6
5	4·3	4·6	8·0	6·1
6	7·3	6·3	9·5	7·7
7	4·8	8·6	8·4	9·1

(i) Meteorological Data

The mean daily maximum and minimum temperatures for the grain-feeding period were 75·0°F (range 58-106°F) and 47·7°F (range 30-78°F) respectively. Little rain fell after the first week of the grain-feeding period until the beginning of the 26th week (5·7 in. in 24 weeks). During the last week 1·9 in. fell over three successive days and 16·4 in. fell during the 9 weeks' recovery period.

(j) Blood and Serum Analyses, Body Fluids and Liver Vitamin A.

Biochemical data for blood and serum analyses, body fluids and liver vitamin A are recorded as group means in Table 4. Differences between groups in final determinations are shown in Table 5.

There was a general tendency in all groups for the mean haemoglobin, P.C.V. and R.C.C. to be elevated above the initial group values at 12 weeks of all-grain feeding. At 26 weeks the blood from animals in Groups I and II exhibited a marked fall in haemoglobin, P.C.V. and R.C.C. levels as compared with their initial values, whereas the haematological values of Groups III and IV tended to approach the initial levels. The lowest individual values in Groups I and II at 26 weeks were haemoglobin 6·9 and 6·6 g/100 ml; P.C.V. 20·2 and 18·8 per cent.; and R.C.C. 4·0 and 4·3 million respectively. The lowest individual values in Groups III and IV were haemoglobin 10·8 and 9·7 g/100 ml; P.C.V. 32·5 and 28·3 per cent.; and R.C.C. 6·8 and 5·8 million respectively.

Group means, standard errors and significant differences of haemoglobin and P.C.V. of the animals after 6 weeks' recovery on pasture following 26 weeks of grain feeding are recorded in Table 6. Anomalous results of two animals from Group I decreased the group average for haemoglobin and P.C.V. These two animals, although apparently normal in appearance, had blood haemoglobin levels of 5·9 and and 7·3 g 100 ml and P.C.V. levels of 19·1 and 21·3 per cent. respectively. Apart from these two animals, blood haemoglobin and P.C.V. levels previously depressed in animals from the daily fed Groups I and II had returned to the pre-experimental levels of the groups.

The group mean serum protein level of all animals exhibited a significant decline at 12 weeks and, with the exception of Group I, a significant decline at 26 weeks. This decline in total serum protein level was largely due to the decrease of serum albumin. The mean serum albumin values decreased in Groups I and II to 2·9 and 2·7 g 100 ml at 26 weeks from initial levels of 3·5 and 3·6 g 100 ml. Individual values declined to as low as 2·2 and 2·0 g/100 ml respectively. The lowest individual values obtained in Groups III and IV were 2·9 and 2·6 g 100 ml respectively at the same sampling period.

TABLE 4

Group Means for Blood and Serum Analyses, Body Fluids and Liver Vitamin A of Heifers Fed 3 lb Crushed Sorghum Grain per Head per Day

Group	I†			II			III†			IV		
Period	Initial	At 12 weeks	At 26 weeks	Initial	At 12 weeks	At 26 weeks	Initial	At 12 weeks	At 26 weeks	Initial	At 12 weeks	At 26 weeks
Prothrombin time (sec)	26	28*	20***	26	24	20***	25	25	18***	26	22**	21***
Blood haemoglobin (g/100 ml)	12·1	12·8	9·4***	11·8	13·0*	8·3***	12·8	13·9**	13·3	11·7	12·8*	11·2
P. C. V. (%)	35·9	39·4*	28·7**	34·7	42·9***	24·8***	36·6	45·0***	39·9	34·8	39·3**	33·0
R. C. C. (millions)	7·6	7·4	6·2**	7·6	8·2	5·5***	7·7	8·7**	7·9	7·3	7·8	6·8
Blood inorganic P (mg/100 ml)	5·9	6·0	6·4	5·4	6·1	7·4***	5·4	6·2*	4·9	6·0	6·2	5·4*
Serum Ca (mg/100 ml)	10·1	8·6***	9·3*	10·3	9·2**	9·3**	9·6	9·0	10·4*	9·8	9·6	10·6*
Serum Mg (mg/100 ml)	2·5	2·4	2·5	2·7	2·5	2·7	2·6	2·7	3·0*	2·4	2·4	2·2
Total serum protein (g/100 ml)	7·2	6·4**	6·8	7·3	6·1***	6·2***	7·1	6·1***	6·5**	7·1	6·5**	6·7*
Serum albumin (g/100 ml)	3·5	2·7***	2·9***	3·6	2·8***	2·7***	3·5	3·0***	3·3	3·6	3·1***	3·1***
Serum globulin (g/100 ml)	3·6	3·6	3·9	3·7	3·3	3·6	3·6	3·1	3·2*	3·5	3·4	3·6
Plasma Cl (m-equiv./l.)	102	97***	104	103	99**	107·5**	102	100*	104	102	97***	103
Plasma Na (m-equiv./l.)	144	155***	139*	144	148	139*	142	147	138*	143	153***	135***
Plasma K (m-equiv./l.)	5·5	4·9**	5·0*	5·1	4·9	5·4	4·9	4·8	4·5	5·1	4·4**	4·5**
Liver vitamin A (μg/g)	140	..	56***	176	..	95**	135	..	68**	184	..	113***
Plasma volume (l.)	11·3	..	8·2*	12·9	..	9·2**	11·8	..	10·3	13·3	..	8·4***
Plasma volume (ml/kg)	47·9	..	51·1 (45·8)	53·9	..	54·3 (48·8)	49·3	..	64·9** (55·7)	56·2	..	56·2 (46·7*)
Blood volume (l.)	17·4	..	10·9***	19·2	..	12·1***	18·4	..	16·8	19·9	..	12·3***
Blood volume (ml/kg)	73·6	..	68·2 (61·0)	80·3	..	71·5 (64·2*)	77·4	..	105·7*** (90·8*)	84·4	..	82·5 (68·6*)
Extracellular fluid volume (l.)	104	..	58**	101	..	61***	110	..	53***	105	..	58***
Extracellular fluid volume (l./100 kg)	43·6	..	34·6*** (30·8***)	41·4	..	35·3*** (31·6***)	46·4	..	32·1*** (28·0***)	45·5	..	38·7*** (32·2***)

† In Groups I and III one animal died and adjustments were made by missing plot technique.
* Significantly lower or higher than initial value at 5% probability level.
** Significantly lower or higher than initial value at 1% probability level.
*** Significantly lower or higher than initial value at 0·1% probability level.

Figures in parentheses are calculated on the basis of body-weights of the animals after the first week on pasture following 26 weeks on restricted grain feeding. This may partially eliminate differences in gastro-intestinal fill.

TABLE 5

Group Means, Standard Errors of Means and Significant Differences for Blood and Serum Analyses, Body Fluids and Liver Vitamin A Obtained at 26 Weeks from Heifers Fed 3 lb Crushed Sorghum Grain per Head per Day

Determination	Means and Standard Errors of Groups				Significant Differences at		
	I†	II	III†	IV	5% level	1% level	0·1% level
Prothrombin time (sec)	20·3 ± 0·4	20·0 ± 0·4	18·3 ± 0·4	21·3 ± 0·4	IV > II	I, II > III	IV > III
Blood haemoglobin (g/100 ml)	9·4 ± 0·6	8·3 ± 0·5	13·3 ± 0·6	11·2 ± 0·5	III > IV IV > I		III > I, II IV > II
P. C. V. (%)	28·7 ± 1·8	24·8 ± 1·7	39·9 ± 1·8	33·0 ± 1·7		III > IV IV > II	III > I, II
R. C. C. (millions)	6·2 ± 0·3	5·5 ± 0·3	7·9 ± 0·3	6·8 ± 0·3	III > IV	IV > II	III > I, II
Blood inorganic P (mg/100 ml)	6·4 ± 0·3	7·4 ± 0·2	4·9 ± 0·3	5·4 ± 0·2		II > I I > IV	II > IV, III I > II
Serum Ca (mg/100 ml)	9·3 ± 0·3	9·3 ± 0·3	10·5 ± 0·3	10·6 ± 0·3		IV, III > II, I	
Serum Mg (mg/100 ml)	2·5 ± 0·1	2·7 ± 0·1	3·0 ± 0·1	2·2 ± 0·1		III > I II > IV	III > IV
Total serum protein (g/100 ml)*	6·8 ± 0·2	6·2 ± 0·2	6·5 ± 0·2	6·7 ± 0·2	I, IV > II		
Serum albumin (g/100 ml)	2·9 ± 0·1	2·7 ± 0·1	3·3 ± 0·1	3·1 ± 0·1	III > I	IV > II	III > II
Serum globulin (g/100 ml)*	3·9 ± 0·1	3·5 ± 0·1	3·2 ± 0·1	3·6 ± 0·1	I > II IV, II > III		I > III
Plasma Cl (m–equiv./l.)	104·1 ± 1·1	107·5 ± 1·0	103·9 ± 1·1	103·2 ± 1·0	II > I, III	II > IV	
Plasma Na (m–equiv./l.)	139·2 ± 1·2	138·6 ± 1·2	138·2 ± 1·2	134·5 ± 1·2	I, II, III > IV		
Plasma K (m–equiv./l.)	5·0 ± 0·2	5·4 ± 0·2	4·5 ± 0·2	4·3 ± 0·2		II > III I > IV	II > IV
Liver vitamin A (μg/g)*	67·6 ± 16·5	84·5 ± 18·2	82·8 ± 16·8	97·8 ± 16·9			
Plasma volume (l.)	8·2 ± 0·6	9·2 ± 0·5	10·3 ± 0·5	8·4 ± 0·5	III > IV, I		
Plasma volume (ml/kg)	51·1 ± 3·0 (45·8 ± 2·6)	54·3 ± 2·7 (48·8 ± 2·3)	64·9 ± 2·7 (55·7 ± 2·3)	56·2 ± 2·7 (46·7 ± 2·3)	III > IV, II (III > II, IV, I)	III > I	
Blood volume (l.)	10·9 ± 0·9	12·1 ± 0·9	16·8 ± 0·9	12·3 ± 0·9		III > IV, II	III > I
Blood volume (ml/kg)	68·2 ± 4·3 (61·0 ± 4·0)	71·5 ± 3·8 (64·2 ± 3·5)	105·7 ± 3·8 (90·8 ± 3·5)	82·5 ± 3·8 (68·6 ± 3·5)	IV > I	III > IV	III > II, I (III > IV, II, I)
Extracellular fluid volume (l.)	57·5 ± 3·8	60·6 ± 3·3	52·9 ± 3·3	57·8 ± 3·3			
Extracellular fluid volume (l./100 kg)	34·6 ± 1·9 (30·8 ± 1·6)	35·3 ± 1·7 (31·6 ± 1·6)	32·1 ± 1·7 (28·0 ± 1·6)	38·7 ± 1·7 (32·2 ± 1·6)			

† In Groups I and III one animal died and adjustments were made by missing plot technique.

* In the case of these variates, final values were significantly dependent on initial values and adjustments made by co-variance analysis.

Figures in parentheses are calculated on the basis of body-weights of the animals after the first week on pasture following 26 weeks on restricted grain feeding.

Although there were statistical differences within groups at different sampling periods and between groups at 26 weeks in levels for prothrombin time, blood inorganic phosphate, serum calcium and magnesium, plasma chloride, sodium and potassium, these levels did not indicate a pathological change at any sampling period and their implications at this stage are not known.

There was a decrease in liver vitamin A reserves in all groups at 26 weeks. Although final group means were significantly dependent on initial group means, individual animals showed considerable variation in the degree of depletion. The lowest level recorded in any animal at 26 weeks was 1·5 μg/g liver in a heifer from Group III. It had an initial level of 112 μg/g liver. Another animal from the same group had an initial level of 198 μg/g liver, which only decreased to a level of 130 μg/g. During the 26th week of grain feeding, the animals were individually driven through a maze at dusk in an endeavour to test for any possible night blindness. All animals negotiated the race successfully.

TABLE 6

Group Means, Standard Errors of Means and Significant Differences of Haemoglobin and P.C.V. of Heifers after Six Weeks' Recovery on Pasture

Determination	Means and Standard Errors of Groups			
	I	II	III	IV
Haemoglobin (g/100 ml)* ..	10·4 ± 0·2**	12·0 ± 0·3	12·3 ± 0·3	12·6 ± 0·2
P.C.V. (%)	30·7 ± 1·4**	34·8 ± 1·3	37·2 ± 1·4	35·6 ± 1·4

* Values adjusted for regression on initial values.

** Significantly lower at 1% probability level than initial values (due to anomalous results of two animals in this group).

Values for blood volume, plasma volume, and E.C.F.V. are expressed as total volume and as a function of total body-weight. Initial body-weight of the animals from pasture would include a greater gastro-intestinal fill than the weight after 26 weeks of grain feeding. Due to this difference, strict comparisons related to body-weight cannot be made between initial values for body fluids and values at 26 weeks. In view of this discrepancy, the body fluid data at 26 weeks have been expressed as a function of the body-weight both at the time of determination and after one week on pasture.

At the end of the grain-feeding period, animals in Group III had a significantly greater blood and plasma volume when compared with animals in the other three groups. There were no significant between-group differences in E.C.F.V. at this time.

VI. DISCUSSION

This experiment has indicated that 3 lb crushed sorghum grain supplemented with 1 per cent. limestone is a satisfactory drought fodder for maiden heifers confined in small yards. These findings are in agreement with the preliminary

data of Morris (1958*b*) and the results obtained by Southcott (1959) with crushed and whole wheat. Mortality under our conditions was low (5 per cent.) and occurred only during the first 16 weeks of all-grain feeding.

Although the animals lost considerable weight during the grain-feeding period of 26 weeks, the rate of weight loss declined as the experiment progressed. During the last 14 weeks, the maximum weight lost in any group was only 2·7 lb per head per week, compared with 7·6 lb per head per week for the first 12 weeks. All groups rapidly regained their lost weight when returned to pasture. The ability of young cattle to gain weight rapidly following transfer from sub-maintenance to productive rations is in agreement with results obtained by other workers (Waters 1908; Winchester and Howe 1955; Morris 1958*b*; Pope 1959).

There were no significant differences between groups in the body measurements after 26 weeks of grain feeding. Height at withers, the more reliable of the measurements for skeletal change used, showed that animals increased slightly in height during the experiment despite a marked loss in body-weight. Although no comparisons were made between the experimental animals and comparable cattle on a high plane of nutrition, the data on increase in wither height with age quoted by Brody (1945) for Jersey and Holstein cattle would suggest that there had been some retardation in skeletal growth. Statistical analyses indicate that chest girth and body-weight in cattle are particularly well correlated (Brody 1945) and our findings of a significant decrease in heart girth, a measurement similar to chest girth, are therefore to be expected.

Franklin and Sutton (1952) and Franklin, McClymont, Briggs, and Campbell (1955) reported that weekly feeding of groups of Merino sheep at levels sufficient for survival was more satisfactory than daily feeding at the same level. Under the conditions of this experiment, twice-weekly feeding of heifers appeared better than either daily or weekly feeding at the rate of 3 lb crushed sorghum grain per head per day. The superiority of the animals fed twice weekly was evident in their general appearance and activity at the conclusion of grain feeding. These animals also had higher body-weights (not significant) at the conclusion of grain feeding and after 9 weeks on pasture.

There was a marked lowering in blood haemoglobin concentration, P.C.V., R.C.C., and serum albumin in the daily-fed groups at 26 weeks. This did not occur in the intermittently-fed groups, although some fall in serum albumin was evident. These markedly lower values in the daily-fed groups are probably a consequence of the greater stress imposed by this system of drought feeding.

In a comparison of the two groups of intermittently-fed animals, those fed twice-weekly had a significantly higher haemoglobin concentration, P.C.V. and R.C.C. at the conclusion of grain feeding. This group also had the greatest total blood volume as determined by the Evans Blue technique.

No apparent metabolic disturbances occurred when animals were introduced to either daily or twice-weekly feeding. However, after the first feed the weekly-fed animals exhibited lethargy and inappetence at the next feed. Probably this would not have occurred with a more gradual introduction to weekly feeding.

After the first week of the experiment, a comparison of the time required for all the feed to be consumed under the different systems of feeding indicated that animals fed weekly had a better opportunity to obtain an equal share of the ration than those fed daily. For this reason, weekly feeding may have greater superiority over daily feeding either when animals vary greatly in age and weight or when there is a shortage of trough space.

In comparing intermittent with daily feeding of groups of sheep under simulated drought-feeding conditions, Franklin and Sutton (1952) and Franklin *et al.* (1955) noted a greater distribution in the body-weight change, expressed as percentage of initial body-weight, of sheep fed daily than of those fed weekly. A similar difference was not observed in this experiment with cattle. The smaller numbers per group, low incidence of "shy" feeders, adequate trough space and uniformity of the animals may have accounted for the smaller distribution in body-weight change.

In practice, twice-weekly feeding would require less labour than daily feeding, and because the feed is consumed immediately it would not be available to predators such as birds and rodents. This is likely to occur with weekly feeding, where uneaten grain would be exposed for 3-4 days each week.

Franklin *et al.* (1955) reported that supplementation of an all-wheat maintenance ration with 0·5 per cent. sodium chloride did not affect body-weight changes or eventual losses in weaner Merino sheep. In this experiment with cattle, there was no advantage in including 0·5 per cent. sodium chloride in the sub-maintenance ration, despite the low levels of sodium and chloride in the sorghum grain. Animals fed daily on this ration lost less weight during the grain-feeding period than animals fed either daily or intermittently without additional salt, but the presence of oedema in six of these animals, together with their slower subsequent rate of weight gain on pasture, could indicate that a greater E.C.F.V. may have been responsible for the difference in body-weights. Although the absolute amount of E.C.F.V. as determined by the thiocyanate technique was greatest in this group, the differences between groups did not reach significance. Meyer (1954) showed an increased E.C.F.V. in growing rats fed diets low in protein and containing very high levels of sodium chloride. The influence of sodium chloride on cattle on a low plane of nutrition is being investigated further, because underground waters in many areas in Queensland contain appreciable levels of total solids, which may be increased during drought times by evaporation.

The mean serum total protein and albumin level was lowest in animals fed 0·5 per cent. sodium chloride daily. This was the only group of animals showing slight subcutaneous oedema of the submandibular space during the last few weeks

of grain feeding. The animal with the lowest serum albumin level (2·0 g/100 ml) in the salt-fed group had the most marked oedema. Another animal in this group with a serum albumin level of 2·9 g/100 ml showed slight oedema. However, there were other animals with serum albumin levels of this order or less in which no oedema was detected, viz. three animals in the salt-fed, five in the daily-fed, one in the twice-weekly fed, and three in the weekly-fed group. The product of plasma volume and total serum protein concentration and also of plasma volume and serum albumin did not indicate that oedema was the result of a deficiency of circulating protein. It is possible that the additional dietary sodium intake of animals in Group II allowed increase of the extracellular fluid and resulted in reduction in protein concentration.

The high incidence of geophagia, as judged by observation of the animals and analysis of the rumen content of one of the animals that died, probably resulted in re-ingestion of much of the undigested grain present in the faeces. Although boredom may have been a factor in the high consumption of earth, the apparent lower incidence in the groups fed intermittently would indicate that other factors are involved. No clinical abnormalities in the animals could be attributed to the high consumption of earth.

The presence of numerous hair-balls in one of the animals that died may indicate that fatal intestinal obstruction could occur with restricted feeding of a ration of grain alone. The occurrence of a large number of hair-balls may be associated with the lack of rumination. Hutyra, Marek, and Manninger (1946) reported their occurrence in cattle, particularly during the suckling period, presumably before rumination commences, and also when cattle are on soft feed. However, limited numbers of hair-balls are sometimes found at autopsy in the rumen of cattle fed rations containing high proportions of roughage.

The facilities used in this experiment did not permit the measurement of water consumption of the individual groups. However, in subsequent studies (unpublished) under comparable temperature conditions, heifers housed in stalls, with *ad lib.* water supply and fed at the same rate of sorghum grain daily, consumed an average of less than 1 gal of water per head per day. This low water consumption is in agreement with the general statement that restriction of feed intake in cattle decreases water consumption (Leitch and Thomson 1944).

It is not possible in drought-feeding experiments of this nature to simulate the environmental conditions pertaining to droughts. The grain feeding commenced in early winter and continued until early summer. In Queensland, with a predominantly summer rainfall, this is the most likely period of nutritional stress in grazing animals. Rain, the necessary factor for termination of a drought, fell in moderate amounts (1·9 in.) during the last week of grain feeding, when it could be expected that the experimental heifers would be weakest and therefore most susceptible to environmental changes. However, this amount of rain, which fell over three successive days, did not appear to have any adverse effect on the animals.

The trends in many of the biochemical values as judged by comparison of initial values with those at 12 weeks of grain feeding were not maintained at 26 weeks. Blood haemoglobin, P.C.V., R.C.C., and plasma sodium levels at 12 weeks were higher than initial values, but this trend was not maintained at 26 weeks. Plasma chloride levels at 12 weeks were lower than initial values; however, the highest levels were recorded at 26 weeks. The reasons are not readily apparent, especially when considering the possible inter-relationships of the components analysed. However, the findings indicate that in this type of experiment such values are dependent on the duration of the experiment and possibly on seasonal variations.

At the conclusion of grain feeding, blood inorganic phosphate levels were lowest in animals fed twice weekly, followed by animals fed weekly, daily, and daily with 0·5 per cent. sodium chloride. There was also an inverse relationship between blood inorganic phosphate and P.C.V. levels. As inorganic phosphate was determined on whole blood, differences between groups might be related to differences in P.C.V. K. W. Moir (personal communication) found that for dairy heifers inorganic phosphate levels in whole blood were consistently lower than levels in plasma, but differences ranged only from 0·5 to 1·0 mg P/100 ml. As these differences are small, it is considered that the appreciable difference in blood inorganic phosphate levels between groups at the conclusion of grain feeding cannot be entirely due to a decrease in P.C.V.

Page, Erwin, Varnell, and Roubicek (1958) fed four groups of yearling heifers an essentially carotene-free ration. Liver samples obtained by biopsy at 33 and 61 days showed that, within groups, the magnitude of hepatic vitamin A expenditure was directly related to the initial amount of vitamin A in the liver (170-50 μg/g). Our results show that after 25 weeks on a ration containing negligible carotenoids, final group means of liver vitamin A (133-56 μg/g) were significantly dependent on initial group means (184-135 μg/g). However, individual animals showed considerable variation in the degree of depletion, and of the 20 animals selected for sampling one had a low (0-2 μg/g) and two others a marginal (2-10 μg/g) liver vitamin A reserve. The levels of these two animals at 26 weeks were not dependent on initial levels. From the absence of night blindness, it is apparent that clinical vitamin A deficiency did not occur in these cattle. In general, the need for vitamin A supplementation will be related to the class of cattle, initial reserves, and the duration of feeding rations containing negligible amounts of carotenoids.

Plasma volume was estimated by determining the optical density of a single sample of plasma taken 20 min after injection of Evans Blue. Subsequent studies of this method were applied to cattle of comparable weight. The rate of disappearance of the dye was plotted by taking readings at approximately 10, 15, 20 and 25 min after injection. Extrapolation of the disappearance curve to zero time indicates that the 20 min reading gave results on the average 11·3 per cent.

too high. However, as the value obtained for the 20 min reading falls on the linear range of the curve, results may be taken as sufficiently comparative for the purpose of this paper.

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NUTRITION OF GRAZING CATTLE

1. Estimation of Protein in Pasture Selected by Grazing Cattle

By K. W. MOIR, B.Sc.*

SUMMARY

A technique for sampling pasture which is representative of pasture selected by grazing cattle is described.

Linear regressions of protein percentage in pasture sampled by this technique on protein percentage in organic-matter faeces are developed for six pasture types.

A logarithmic regression is fitted to data from all six pasture types. Data derived from this regression agree satisfactorily with similar data calculated from published digestibility trials on pasture grasses.

Based on the protein content in organic-matter faeces, the regression equations allow an assessment of the protein content of the pastures selected by grazing cattle.

I. INTRODUCTION

Raymond, Kemp, Kemp, and Harris (1954) discussed the difficulty in obtaining samples representative of pasture selectively grazed. During the course of digestibility studies in which samples of herbage similar to that actually grazed were required, these workers suggested that the composition of faeces produced by grazing stock might be used as an indirect measure of the composition of the herbage grazed. They showed a relationship between the nitrogen content of herbage grazed and that of the resulting faeces.

The object of the investigations reported in the present paper was to examine the relationship between protein content of pastures selected by grazing cattle and protein content of the resulting faeces.

II. EXPERIMENTAL

(a) Area Investigated

Seventy-four dairy properties were examined. These were located in south-eastern Queensland in an area extending 60 miles north and south and 30 miles west of Brisbane. Only those farms on which unimproved pasture was the only feed available were considered in this study. The farms ranged in size from 50 to 400 ac.

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(b) Methods of Sampling

(i) Pasture Sampling

The aim was to obtain grass samples considered to be representative of the diets selected by grazing cattle.

Paddocks to which the herd had access during the four days prior to investigation were sampled. Within paddocks, areas of pasture of at least half an acre obviously different from the remainder of the paddock were considered separately. This procedure ensured that all pasture species grazed could be represented in the sample.

In sampling, sub-samples were taken from adjacent ungrazed grasses at the height to which the same species at a similar stage of growth had been cropped. At each point the area of sampling was 9 sq. in. All sub-samples from the one farm were bulked for analysis.

The distance traversed when sampling in small paddocks was 50 yd for each acre. One sub-sample was taken within every 10 yd but only if there were obvious signs of grazing within that length. In large paddocks the distance traversed was 5 yd for each acre. One sub-sample was taken within every yard but only if there were obvious signs of grazing within the yard length. One typical sub-sample was taken in areas of less than half an acre.

On each farm at each sampling the pasture was composed predominantly of one grass species, except in the case of forest grass, which was a mixture of a number of species usually referred to as "forest grass". The pastures are classified into six types according to the species predominant in the selected diet. These species were:—

A Narrow-leaf carpet grass (*Axonopus affinis*)

C Blue couch grass (*Digitaria didactyla*)

F Forest grasses

Forest blue grass (*Bothriochloa intermedia*)
Pitted blue grass (*Bothriochloa decipiens*)
Kangaroo grass (*Themeda australis*)
Wire grasses (*Aristida* spp.)
Bunch spear grass (*Heteropogon contortus*)

R Rhodes grass (*Chloris gayana*)

P Paspalum (*Paspalum dilatatum*)

K Kikuyu grass (*Pennisetum clandestinum*)

(ii) Sampling of Faeces

As the number of faecal sub-samples within each herd must be sufficient to equalize differences in metabolism of protein and selective grazing habits of individual cows, the number of sub-samples required to give a sample representative of the total faeces from each herd was first determined. For this determination faecal samples were taken from two groups of 10 cows in each of 20 herds. From each group bulked samples comprising 150 g wet-weight faeces from each cow were analysed for protein and ash. The standard error of means of percentage protein in faeces expressed on an organic-matter basis for samples representing 10 and 20 animals are:—

—	D.F.	S.S.	M.S.
Between farms	19	83·50	4·395
Between duplicates	20	2·08	0·104

Whence the standard error of the means for 10 samples = 0·32 per cent. protein; and the standard error of the means for 20 samples = 0·23 per cent. protein.

Comparisons were made between the protein content of bulked faecal samples comprising 150 g wet-weight faeces from individual cows and the protein content of samples taken from freshly voided faeces in the field. Agreement was satisfactory. In the latter procedure about 25 g wet-weight faeces were taken from at least 10 and preferably from 20 faecal pads and the samples from each herd were bulked for analysis. This procedure was subsequently adopted in this study.

(c) Methods of Analysis

Samples were oven-dried at 100°C and milled.

Ash was determined gravimetrically on 2 g aliquots ashed at 600°C for 1 hr.

The method of analysis for protein was essentially that of the Association of Official Agricultural Chemists (1950) with a modification of the catalysts used in digestion. About 10 mg each of copper sulphate and selenium were used.

In 74 pasture samples examined, the mean ash content was 9·2 per cent. ash and the range 6·8 to 11·9 per cent. In the 74 resulting faecal samples the mean ash content was 17·5 per cent. ash and the range 11·5 to 25·8 per cent. The variations in ash contents of the pasture and faecal samples are sufficiently wide to introduce errors in the study of the relationship between protein percentage in pasture and in faeces, expressed on a dry-matter rather than on an organic-matter basis. However, as the nutritive values of feeds are usually compared on a dry-matter basis, this method of expression was used in calculating protein percentage in pastures. Errors due to variation in ash content of faeces were eliminated by expressing protein in faeces on an organic-matter basis.

III. RESULTS

(a) Relationship Between Protein in Pasture and in Faeces

Percentages of protein in dry-matter pasture and in organic-matter faeces are plotted in Figure 1. From inspection of the distribution of variates in Figure 1, linear regression appears to adequately fit the data.

Details of statistical analyses of data from the six pasture types represented in Figure 1 are given in Table 1.

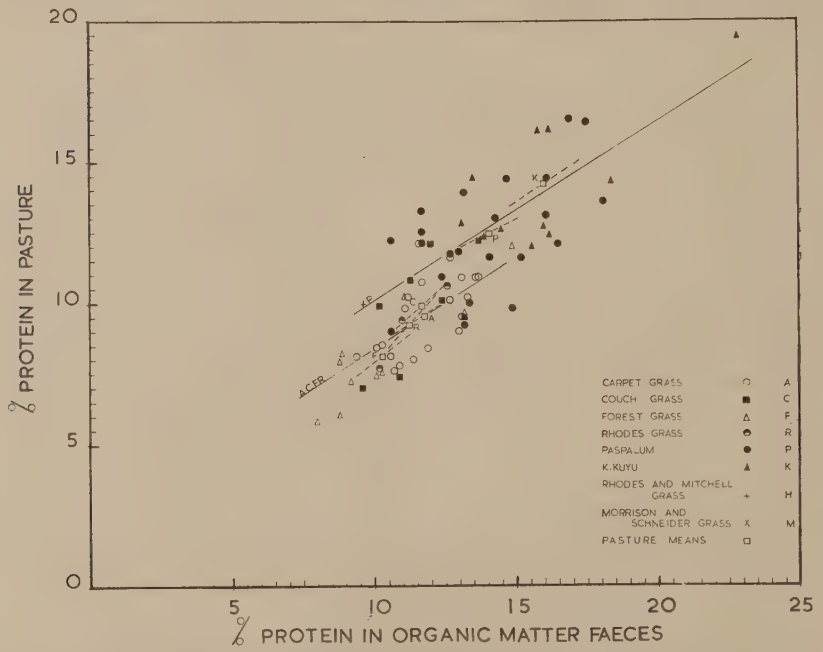

Fig. 1.—Relationship between protein in dry-matter pasture and in organic-matter faeces. Linear fitting to data from this study.

Although faecal protein content is obviously biologically dependent on feed it has been treated statistically in Table 1 as the independent variable. This is because faeces are almost certainly less affected by sampling errors than are pastures. There is no evidence of heterogeneity among the six residual mean squares (X^2 for 5 D.F. : 6·1 corresponding to a probability of about 0·3) or among the slopes of the six regression lines:—

—	D.F.	M.S.
Average regression	1	110·141
Among regressions	5	0·746
Residual	62	1·985

However, there are significant differences among the pasture means:—

—	D.F.	M.S.
Among pastures (adjusted for regression)	5	6·002 ($P < 0·05$)
	67	1·893

Adjusted means—

A	10·21
C	10·64
F	9·79
R	10·24
P	11·67
K	12·18

There are no significant differences within the brackets but highly significant differences between the two bracketed groups.

From the above analyses, it appears that the appropriate estimating equation must include an adjustment at least for the two groupings, if not for each of the six pastures. These estimating equations take the form:

$$y = 1·9 + 0·64\ x \pm 1·4 \text{ (for pastures A, C, F, R)}$$

$$y = 3·6 + 0·64\ x \pm 1·4 \text{ (for pastures P, K)}$$

The differences among pasture means may represent not real differences among pastures but rather errors in sampling. If this is so, the slope of the line is unaffected but choice of the y intercept value would have to be arbitrary.

TABLE 1

Statistical Analysis of the Relationship between Protein in Dry-matter Pasture and in Organic-matter Faeces

Predominant Grass in Diet	No. of Samples	Mean of y's* (%)	Mean of x's† (%)	Regression Coefficient	Residual Mean Square
Carpet (A) ..	20	9·54	11·81	0·682	1·2120
Couch (C) ..	8	9·88	11·66	0·877	2·4690
Forest (F) ..	10	8·17	10·33	0·785	0·8857
Rhodes (R) ..	3	9·23	11·27	1·143	0·3459
Paspalum (P) ..	22	12·43	14·03	0·531	2·8827
Kikuyu (K) ..	11	14·20	16·00	0·628	2·3765
Within grass types				0·642	1·8930

* y = Protein percentage in pasture.

† x = Protein percentage in organic-matter faeces.

(b) Extrapolation and Comparison with Published Data

Digestibility data derived on Rhodes grass and Mitchell grass of low protein content (Harvey 1952) and on green pastures of moderate to high protein contents (Morrison 1937; Schneider 1947) were also used in deriving the relationship between protein in pasture and in organic-matter faeces. Percentages of protein in dry-matter pasture and in organic-matter faeces, together with similar data calculated from published trials, are given in Figure 2.

Comparison of linear fitting to the three groups of data (Morrison and Schneider, Harvey and this study) is shown in Table 2.

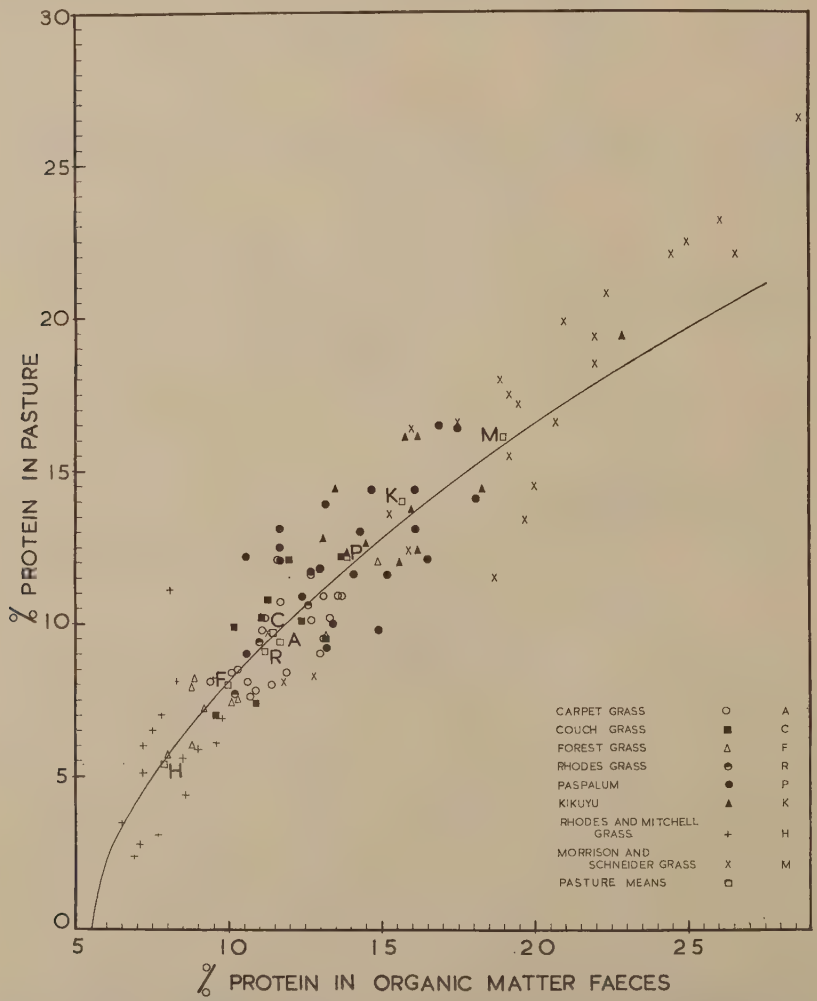

Fig. 2.—Relationship between protein in dry-matter pasture and in organic-matter faeces. Logarithmic fitting to data from this study and from published digestibility data.

TABLE 2

Comparison of Linear Fitting to Data from Morrison and Schneider, Harvey and this Study

Data from	No. of Samples	Mean of y's	Mean of x's	Regression Coefficient	Residual Mean Square
Morrison, Schneider ..	24	16·84	19·78	0·984	3·40
Harvey	16	5·79	8·08	1·089	4·26
This study (within pastures)	..	..	..	0·642	1·89

The differences among the three residual mean squares approach the 5 per cent. level of significance (X^2 for 2 D.F. : 5·7) and the slopes are significantly different at the 5 per cent. level:—

—	D.F.	M.S.
Average regression	1	568·601
Among regressions	2	10·405
Residual	103	2·535

From inspection of Figure 2 it appears that all data are related logarithmically. At zero protein intake a curve relating protein in pasture and in faeces would intersect the abscissa at a point equivalent to metabolic faecal protein. Blaxter and Mitchell (1948) found that metabolic faecal nitrogen was 0·5 g nitrogen per 100 g dry-matter intake.

To convert metabolic nitrogen to a percentage in organic-matter faeces requires a knowledge of the dry-matter digestibility of the pasture and the organic matter content of faeces. From examination of data presented by Harvey (1952) on Rhodes grass and Mitchell grass of negligible protein content, the dry-matter digestibility at a hypothetical zero protein level in pasture would be of the order of 30 per cent. On the basis of 82 per cent. organic matter in faeces, metabolic faecal protein would be equivalent to about 5·5 per cent. protein.

A logarithmic curve of the form $\log y = a + b \log (x - 5{\cdot}5)$ is therefore indicated as appropriate to the data. Statistical analyses of this form fitted to the data derived from six pasture types in south-eastern Queensland are given in Tables 3 and 4.

TABLE 3

Logarithmic Fitting to Six Pasture Types

Pasture Type	No. of Samples	Mean log y	Mean log (x − 5·5)	Regression Coefficient	Residual Mean Square	Mean y	Mean x
A	20	0·975	0·791	0·445	0·002418	9·44	11·68
C	8	0·987	0·779	0·581	0·005007	9·71	11·51
F	10	0·902	0·649	0·487	0·002638	7·98	9·96
R	3	0·961	0·754	0·747	0·000765	9·12	11·18
P	22	1·089	0·917	0·325	0·003866	12·26	13·76
K	11	1·148	1·010	0·448	0·002398	14·05	15·72
Within pasture types				0·439	0·003010	..	..

TABLE 4

Components of Variance and Co-variance for Among, Within and Among plus Within Pasture Types

	Components of Variance and Co-variance		
	log y	Co-variance	log (x − 5·5)
Among	0·007791	0·010787	0·014532
Within	0·005576	0·005943	0·013531
Among + Within ..	0·013368	0·016730	0·028064

From these data the regression estimate from the among plus within line is calculated as—

$$\log y = 0{\cdot}52349 + 0{\cdot}59614 \log (x - 5{\cdot}5)$$

i.e. $$y = 3{\cdot}34\ (x - 5{\cdot}5)^{0{\cdot}60}$$

Variations about this line can be ascribed to two independent sources:—

(1) Variation among pasture types (either real differences or sampling errors).

(2) Variation among samples of the same pasture type.

It is difficult to attach a standard error of estimate to this relationship. In particular, the "among pasture" components are not well determined from only six pasture types. However, it appears that this line fits the data at least as well as the linear regression. In Figure 3 is shown the distribution of variates about the line resulting from plotting values on logarithmic paper.

Results of the logarithmic fitting to the data recorded in the literature are given in Table 5.

TABLE 5

Results of Logarithmic Fitting to Data from Morrison and Schneider and Harvey

Data	No. of Samples	Mean log y	Mean log (x − 5·5)	Regression Coefficient	Residual Mean Square	Mean y	Mean x
Morrison, Schneider ..	24	1·207	1·129	0·800	0·003199	16·10	18·9
Harvey ..	16	0·729	0·377	0·599	0·023281	5·36	7·8

The residual mean squares for data of Morrison and Schneider on this transformed scale are close to the "within pastures" residual mean square of Table 3. The residual mean squares for data of Harvey are much higher than the residual mean squares in Table 3, as is to be expected at the lower end of the logarithmic curve.

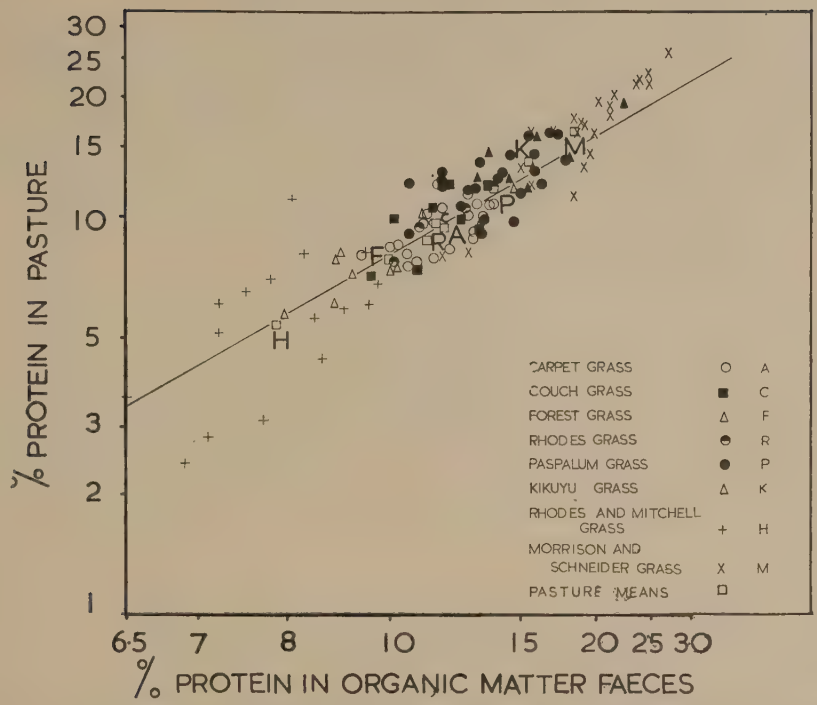

Fig. 3.—Relationship between protein in dry-matter pasture and in organic-matter faeces. Logarithmic fitting to data from this study and from published digestibility data. Plotted on log paper.

TABLE 6

Comparison of Apparent Protein Digestibilities

Protein in Pasture Dry-matter (%)	Apparent Protein Digestibility (%)		
	This Study	Mitchell	Glover *et al.*
25	80·4	81·2	83·5
20	76·7	76·3	76·8
15	71·1	70·0	68·1
10	61·5	60·3	55·8
5	35·1	0	34·8

Percentages of protein in pasture and in faeces may be used to calculate apparent protein digestibility when the feed-to-faeces ratio is known. Lancaster (1954) derived an equation for estimating the feed-to-faeces ratio on an organic-matter basis from protein percentage in organic-matter faeces. Using the Lancaster estimating equation as a functional relationship to derive the feed-to-faeces ratio

from faecal protein and the logarithmic curve of this study relating pasture and faecal protein as a functional relationship, together with correction for an average ash content of 9·0 per cent. ash in pasture, the apparent protein digestibilities at various levels of protein in pasture were calculated. These apparent protein digestibilities are compared in Table 6 with those calculated from equations relating apparent protein digestibility to protein percentage in forage derived by Mitchell (1942) and Glover, Duthie, and French (1957). These equations are:—

$$y = 42{\cdot}64\ (x - 5)^{0{\cdot}2148} \text{ (Mitchell)}$$

$$\text{and } y = 69{\cdot}7 \log x - 13{\cdot}9 \text{ (Glover } et\ al.\text{)}$$

IV. DISCUSSION

Percentages of protein in the pasture species predominant in the diets of cattle in south-eastern Queensland and percentages of protein in pasture grasses recorded in the literature are similarly related to the percentages of protein in the resulting organic matter faeces by a logarithmic curve. Estimates of protein in pasture over the more linear range of the logarithmic curve derived in this paper agree within 7 per cent. of the percentage protein estimated from the linear regression equation derived by Raymond *et al.* (1954) on grasses used in digestion trials.

In Table 6 there is good agreement of estimates of apparent protein digestibilities betwen those derived in this study and those derived by Mitchell for protein levels between 10 and 25 per cent. protein, and fair agreement with those derived by Glover *et al.* over the whole range of protein levels in pasture. The generally satisfactory agreement supports the validity of the logarithmic curve derived in this study and the Lancaster estimating equation in a broad overall application.

The linear regressions developed for six pasture types in south-eastern Queensland could be used to estimate the order of magnitude of protein percentage in these pastures within the range of protein values of the 74 samples. With other pasture types or towards or outside the limits of these protein values for these pasture types, the logarithmic curve would be preferred.

The uniform distribution of varieties found in this investigation for many different pastures supports the validity of the sampling technique in its overall application.

V. ACKNOWLEDGEMENTS

The field data for this and subsequent investigations reported under the title "Nutrition of Grazing Cattle" were collected in collaboration with Mr. A. McTackett during investigations of infertility in cattle in relation to nutrition. Mr. McTackett's assistance and field experience were of immense value. The author also gratefully acknowledges the assistance of Mr. A. W. Beattie (Biometrician) for statistical analyses.

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NUTRITION OF GRAZING CATTLE

2. Estimation of Phosphorus and Calcium in Pasture Selected by Grazing Cattle

By K. W. MOIR, B.Sc.*

SUMMARY

Linear regressions of phosphorus in pasture samples representative of pasture selectively grazed by cattle on phosphorus in organic-matter faeces are developed for six pasture types.

A linear regression is calculated for the six pasture types.

Estimates of phosphorus percentage from this regression in feeds fed to stalled cattle are compared with analytical values of phosphorus percentage in these feeds. Agreement is satisfactory.

Regressions of calcium in selectively grazed pasture on calcium in organic-matter faeces are developed.

The effect of calcium in drinking water is an appreciable source of error in the regression of calcium in pasture on calcium in organic-matter faeces.

The accuracy of estimates from regression of phosphorus and calcium in selectively grazed pasture is not increased by including other variables in multiple regression analysis.

I. INTRODUCTION

A technique for sampling pasture which is representative of pasture selected by grazing cattle has been described (Moir 1960). A regression of protein percentage in pastures sampled by this technique on protein percentage in organic-matter faeces was developed. Data derived from this regression agreed satisfactorily with similar data calculated from published digestibility trials on pasture grasses. This observation supported the validity of the pasture sampling technique in yielding samples representative of pasture selectively grazed.

In the present investigation of a relationship between pasture and faecal phosphorus and pasture and faecal calcium, the samples of faeces and of pasture from the previous investigation are used. A general relationship could be expected, as phosphorus and calcium excreted by cattle are mainly in the faeces. If estimates of phosphorus and calcium in selectively grazed pasture are to be made using this relationship, it could be expected that adjustments would be necessary for the amounts of these elements required for milk production, gestation and growth.

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During the course of the previous investigation, average milk yields of herds, and in some cases the milk yields of individual cows, were recorded. Milk production is therefore a measured variable in this investigation of the relationship between pasture and faecal phosphorus and calcium. The effect of gestation is largely controlled when milk production is measured. The demands for phosphorus and calcium for foetal development are appreciable only in late gestation. Cows in late gestation are dry cows or low-producing cows in late lactation. Growth is also largely controlled in this investigation, as faecal samples were taken mainly from milking herds and samples from very young animals were therefore excluded.

Another variable considered is dry-matter digestibility, which may exert an effect on phosphorus and calcium content of faeces. As the dry-matter digestibility of pasture grass roughly parallels the protein content of the grass and as a relationship exists between protein in pasture and protein in organic-matter faeces, either pasture protein or faecal protein may be used as a comparative index of dry-matter digestibility. The broad application of the equation derived by Lancaster (1954) for estimating organic-matter digestibility from faecal protein was referred to previously (Moir 1960). This equation may be used for estimating dry-matter digestibility on a more absolute basis.

The procedure of Moir (1960) was used for sampling pasture and faeces.

For the estimation of phosphorus and calcium, the ash from 2 g dry-matter samples of pastures or of faeces was evaporated to dryness with 15 ml concentrated HCl, a further 10 ml concentrated HCl was added, and after warming the volume was made up to 200 ml with distilled water and allowed to stand.

The colorimetric method of the Association of Official Agricultural Chemists (1950) was used to estimate phosphorus on aliquots of the acid-soluble ash.

Calcium was estimated on a semi-micro scale by potassium permanganate titration of precipitated calcium oxalate.

III. EXPERIMENTAL AND RESULTS

The minimum number of faecal sub-samples required to give samples representative of the faeces of the herd in respect to phosphorus and calcium was determined from data derived from samples collected in the previous investigation (Moir 1960). These samples were taken from two groups of 10 cows paired in respect of milk production in each of 20 herds. Percentages of phosphorus and calcium in these samples of faeces were calculated on an organic-matter basis and details of statistical analysis of these data are given in Table 1.

	Phosphorus	*Calcium*
Standard error of means of 10 samples =	0·034	0·071
Standard error of means of 20 samples =	0·024	0·050

TABLE 1

Statistical Analysis of Data from Faecal Samples from Paired Groups of 10 Cows in each of 20 Herds

Source	D.F.	Mean Squares	
		Phosphorus	Calcium
Between farms	19	0·04729	0·33677
Between duplicates	20	0·00117	0·00504

As pairing in respect of milk production is more effective than correcting for milk production, these results under-estimate the standard errors of samples taken from a cross-section of a milking herd. However, these standard errors indicate that at least 20 sub-samples from a herd are preferred.

(a) Relationship of Phosphorus in Selectively Grazed Pasture and in Organic-matter Faeces

Percentages of phosphorus in dry-matter pasture and in organic-matter faeces for the six pasture types previously sampled are plotted in Figure 1. The data appear to be adequately defined by linear regression. Details of the statistical analysis of these data are given in Table 2.

TABLE 2

Statistical Analysis of the Linear Relationship Between Phosphorus in Dry-matter Pasture and in Organic-matter Faeces

Predominant Grass in the Diet	No. of Samples	Mean of y	Mean of x	Regression Coefficient	Residual Mean Square
Carpet grass (A)	20	0·177	0·369	0·291	0·001189
Couch grass (C)	8	0·216	0·447	0·400	0·001490
Forest grass (F)	10	0·159	0·349	0·379	0·001523
Rhodes grass (R)	3	0·303	0·557	0·013	0·000598
Paspalum grass (P) ..	22	0·269	0·587	0·218	0·001371
Kikuyu grass (K)	11	0·315	0·699	0·312	0·002069
Within pasture types				0·295	0·001579

There is no evidence of heterogeneity among the six residual mean square (X^2 for 5 D.F. : 1·2 corresponding to a probability of 0·95) or among the slopes of the six regression lines:—

—	D.F.	M.S.
Average regression	1	0·1366
Among regressions	5	0·0022
Residual	62	0·0014

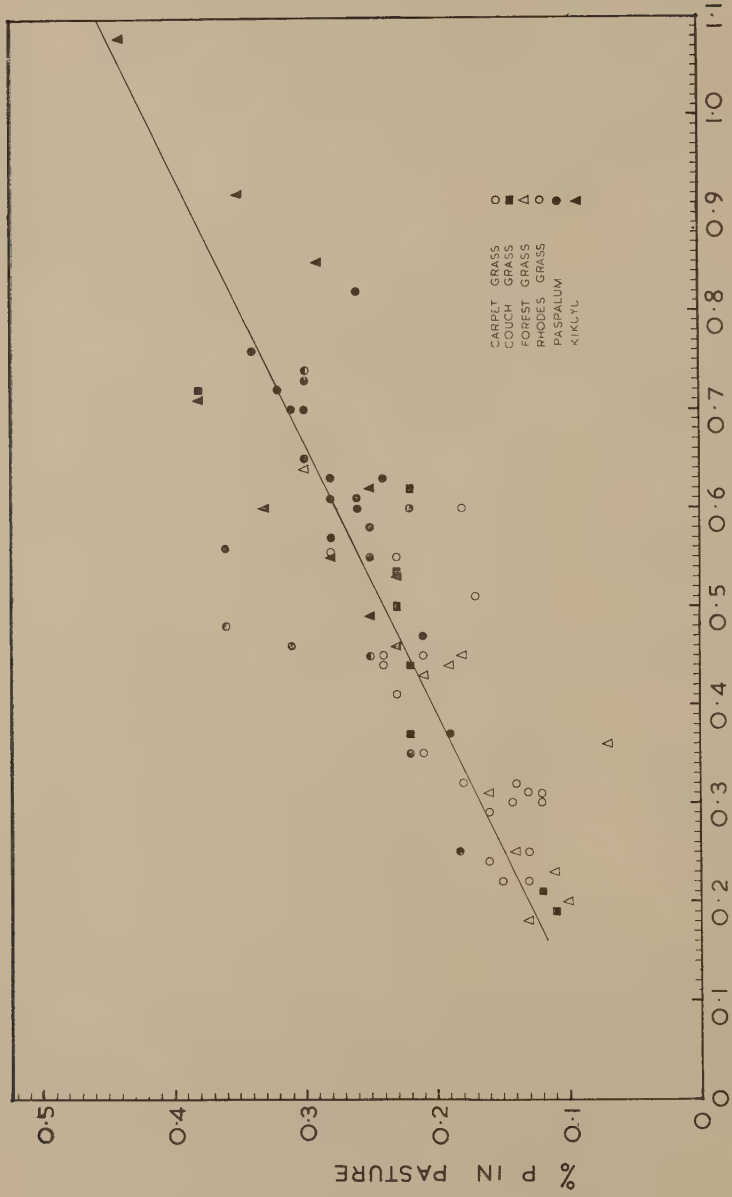

Fig. 1.—Relationship between phosphorus in dry-matter pasture and in organic-matter faeces.

However, there are significant differences among the pasture means:—

—	D.F.	Mean Square
Regression on x_1	1	0·13661***
Residual within pastures	67	0·00158
Pastures adjusted for regression	5	0·00420*

* = significant at the 5% level.
*** = significant at the 0·1% level.

Adjusted Means—

F	0·202
A	0·214
C	0·230
P	0·242
K	0·255
R	0·285

There are no significant differences within the brackets. Other differences are significant at 5 per cent. and 1 per cent. levels.

From the above analyses the estimating equation is—

$$y = p - 0{\cdot}146 + 0{\cdot}295\ x_1 \pm 0{\cdot}04,$$

where p is the appropriate adjusted mean.

If it is assumed that the pasture effects are a sample from a population of pasture effects or sampling errors, an overall relationship can be estimated by component analysis. Details of this analysis are given in Table 3.

TABLE 3

Components of Variance and Co-variance for Among, Within and Among Plus Within Pasture Types

Source	Components of Variance and Co-variance		
	(y^2)	(xy)	(x_1^2)
Among	0·003742	0·008421	0·018722
Within	0·003564	0·006813	0·023111
Among + Within	0·007306	0·015234	0·041833

From these data the regression estimate from among plus within line is calculated as—

$$y = 0{\cdot}057 + 0{\cdot}364\ x_1$$

(b) Relationship of Calcium in Selectively Grazed Pasture and in Organic-Matter Faeces

Percentages of calcium in dry-matter pasture and in organic-matter faeces for six pasture types are plotted in Figure 2. The data appear to be adequately defined by linear regression. Details of the statistical analyses of these date are given in Tables 4 and 5.

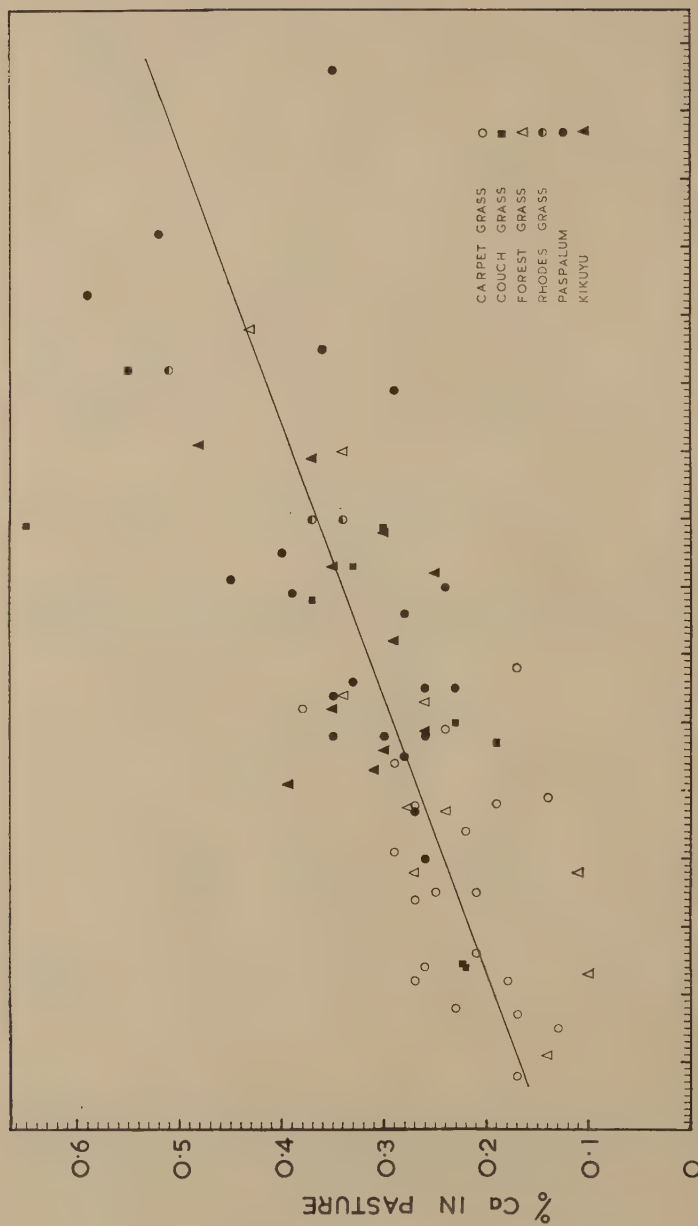

Fig. 2.—Relationship between calcium in dry-matter pasture and in organic-matter faeces.

TABLE 4

Analysis of Variance for Calcium in Pasture and in Organic-matter Faeces

Source	D.F.	Mean Square
Among pastures .. .. (adjusted for regression)	5	0·00217 N.S.
Regression	1	0·22458***
Residual	66	0·00550

N.S. = Not significant.
*** = Significant at the 0·1% level.

TABLE 5

Regression of Calcium in Pasture on Calcium in Organic-matter Faeces

Source	D.F.	Mean Square
Regression on x ..	1	0·43221***
Residual	71	0·00556

*** = Significant at the 0·1% level.

The among pasture differences in Table 4 are not significant. Ignoring pasture differences leads to the analysis in Table 5.

These results lead to the estimate—

$$y = 0{\cdot}092 + 0{\cdot}247x \pm 0{\cdot}074.$$

(c) Effect of Milk Production and Dry-matter Digestibility on Regressions for Phosphorus and Calcium

Several regression forms were fitted in turn, using the sums of squares and products of these variates within the six pasture types. These were:—

(i) $y = a + bx_1 + cx_5$

(ii) $y = a + bx_3$

(iii) $y = a + bx_1 + cx_3$

(iv) $y = a + bx_1 + cx_3 + dx_5$

(v) $y = a + bx_1 + cx_4$

The terminology used is—

y = *per cent.* P or *per cent.* Ca in dry-matter pastures.

x_1 = *per cent.* P or *per cent.* Ca in organic-matter faeces.

x_2 = *per cent.* protein in organic-matter faeces.

x_3 = *per cent.* P or *per cent.* Ca in dry-matter faeces multiplied by the estimated dry-matter indigestibility: i.e. $x_3 = zx_1 (u + vx_2)$, where u and v are constants suggested by Lancaster for estimating feed-to-faeces ratio and hence dry-matter digestibility, and z is an adjustment for the ratio of organic matter to dry matter.

$x_4 = x_1 \cdot x_2$.

x_5 = milk production (herd average in lb).

The residual mean squares were not reduced when these forms were fitted to the calcium data.

Only the form $y = a + bx_1 + cx_3$ significantly reduced the residual mean squares for the phosphorus data. This suggests that the values of u and v used in calculating x_3 are not optimal for this set of data. In this regard Lancaster did find significant differences among what would be called in this analysis his "u" values. Details of the analysis of the form $y = a + bx_1 + cx_3$ are given in Tables 6 and 7.

TABLE 6

Statistical Analysis of the Form $y = a + bx_1 + cx_3$

Source	D.F.	Mean Square
Regression on x_3	1	0·14238***
Extra due to x_1	1	0·00800*
Residual within pastures	66	0·00139
Pastures adjusted for x_3 and x_1 ..	5	0·00305 N.S.

*** = significant at the 0·1% level.
* = significant at the 5% level.
N.S. = not significant.

TABLE 7

Statistical Analyses of the Form $y = a + bx_1 + cx_3$ when Differences Among Pastures are Ignored

Source	D.F.	Mean Square
Regression on x_3	1	0·33961***
Extra due to x_1	1	0·03067***
Residual	71	0·00151

*** = significant at the 0·1% level.

The estimating equation is—

$$y = 0{\cdot}0247 + 0{\cdot}2103x_1 + 0{\cdot}6831x_3 \pm 0{\cdot}039.$$

(d) Comparison of Phosphorus and Calcium Estimated from Regression with Values Derived from Analyses of Feeds

Details of the comparison of phosphorus and calcium estimated from regression with values derived from analyses of feeds fed to stalled animals are summarized in Table 8. The animals recorded were fed for three weeks on each feed. Samples of feeds and faeces were collected twice daily during the second half of each period and analyses were made on bulked samples.

TABLE 8

Comparison of Percentages of Phosphorus and Calcium Estimated from Regression with Values Derived from Analyses of Feeds Fed to Stalled Animals

Animal Identification	Milk (lb)	Feed	Faecal Composition (Organic-matter basis)		Feed Composition Observed (Dry-matter basis)		Feed Composition Estimated (Dry-matter basis)	
			P (%)	Ca (%)	P (%)	Ca (%)	P (%)	Ca (%)
A	0	Oaten hay	0·25	0·38	0·13	0·15	0·15	0·19
B	0		0·28	0·33			0·16	0·17
A	8	Oaten hay	0·28	0·39	0·13	0·16	0·16	0·19
B	6		0·26	0·35			0·15	0·18
A	0	Lucerne hay	0·74	2·46	0·35	1·05	0·33	0·70
B	0		0·76	2·53			0·33	0·72
A	11	Lucerne hay	0·71	2·25	0·36	0·97	0·32	0·65
B	10		0·68	2·24			0·30	0·65
C	21	Lucerne hay	0·83	3·76	0·38	1·54	0·36	1·02
D	13		0·86	3·73			0·37	1·01
C	25	Lucerne hay	0·74	3·61	0·33	1·45	0·33	0·98
D	18		0·72	3·44			0·32	0·94
E	0		0·87	3·86			0·37	1·05

The estimated percentages of phosphorus are within the standard error of estimate for the regression relating pasture and faecal phosphorus. There is unsatisfactory agreement between the estimated and observed percentages of calcium in the feed.

Regression analysis of the observed percentages of calcium in feeds on the percentages of calcium in organic-matter faeces from Table 8 yields the equation

$$y = 0{\cdot}0189 + 0{\cdot}4049\,x \pm 0{\cdot}03$$

IV. DISCUSSION

The linear regression of phosphorus percentage in selectively grazed pasture on phosphorus percentage in organic-matter faeces developed for each of the six pasture types may be used to estimate the order of magnitude of the phosphorus levels in these selectively grazed pastures.

The regression equation developed by component analysis when pasture differences are ignored gave estimates of phosphorus percentage in the diets of stall-fed animals (Table 8) which when compared with the analysed percentages of phosphorus in these diets were within the standard error of 0·04 per cent. phosphorus. The satisfactory application of this regression to an entirely different set of conditions and to different feed types suggests that this regression could be used generally to distinguish low, moderate and high phosphorus levels in the diets of cattle.

The linear regression of calcium in selectively grazed pasture on calcium in organic-matter faeces gave estimates of calcium in the diets of the stall-fed cattle which were considerably less than the analysed percentages of calcium in these diets. Also the slope of the regression of calcium in pasture on calcium in organic-matter faeces is considerably less than the slope of regression of phosphorus in pasture on phosphorus in organic-matter faeces. These observations suggested that calcium in the pasture samples analysed under-estimated the total calcium intake. The possibility that an appreciable amount of calcium was supplied by the drinking water was subsequently investigated. Analyses of water samples from 35 of the farms revealed that the calcium content of these water samples ranged from 0·03 to 1·73 g Ca per gal and the mean was 0·24 g. A high calcium content in water from relatively few farms could appreciably affect the slope of the regression line and could contribute to the high standard error of 0·073 calcium. The high standard error is also possibly due to errors in sampling pastures containing white clover, as the calcium content of white clover may be several times as great as the calcium content of grasses.

The linear regression of calcium in selectively grazed pasture on calcium in organic-matter faeces developed by component analysis for the six pasture types may be used to estimate the order of magnitude of the calcium levels in these selectively grazed pastures. The linear regression of calcium in feeds on calcium in organic-matter faeces developed from data recorded in Table 8 may be used to estimate the order of magnitude of the total ingested calcium.

As the inclusion of milk production and dry-matter digestibility as measured variables in the regression analyses did not improve the accuracy of estimates of phosphorus or calcium in selectively grazed pasture, it must be concluded that their effects on these regressions are small. Small effects of milk production may be explained by (1) the compensating effect of late gestation in low-producing cows; (2) the replacement of depleted body reserves by low-producing cows; and (3) lower feed intakes of low-producing cows.

Small effects due to milk production or dry-matter digestibility on the regression of pasture and faecal phosphorus would be unlikely to be detected because of a correlation between pasture phosphorus and milk production and between pasture phosphorus and dry-matter digestibility. These effects are therefore virtually accounted for in the one linear regression. No conclusions can be made in respect of calcium, as errors in the data used are high.

V. ACKNOWLEDGEMENT

The author gratefully acknowledges the statistical analyses done by Mr. A. W. Beattie (Biometrician in the Department).

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NUTRITION OF GRAZING CATTLE

3. Estimation of Protein, Phosphorus and Calcium in Mixed Diets

By K. W. MOIR, B.Sc.*

SUMMARY

Regressions developed to relate dietary and faecal composition in terms of protein, phosphorus and calcium are applied to estimate the percentages of protein, phosphorus and calcium in diets comprising two or more feeds differing widely in chemical composition.

The regressions for phosphorus and calcium give satisfactory results when applied over a wide range of dietary phosphorus and calcium levels in a variety of feedstuffs.

The regression for estimating protein percentage is unsatisfactory, giving a marked under-estimate of the protein level in most mixed diets.

When the diet consists of two feeds which differ in phosphorus or calcium content, analysis of each feed together with the regression estimates of phosphorus and calcium permits an assessment of the relative proportion of each feed in the diet. From this proportion the protein content in the mixed diet may be calculated.

I. INTRODUCTION

Regressions were developed previously (Moir 1961*a*, 1960*b*) to relate dietary and faecal composition in terms of protein, phosphorus and calcium. These regressions were developed on data from relatively uniform diets.

The object of the investigation reported in the present paper is to determine whether these regressions could be applied to diets comprising two or more feeds differing widely in chemical composition.

II. METHODS

The procedure of Moir (1960*a*) was used for sampling pastures and faeces. The pasture sampling technique was also used for sampling grazed crops.

The analytical procedures described by Moir (1960*a*, 1960*b*) were used.

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III. EXPERIMENTAL AND RESULTS

Samples of feeds and faeces were collected from 11 dairy farms. In Table 1 are recorded:—

(1) Feeding practices at the time of sampling.

(2) Percentages of protein, phosphorus and calcium from analysis of feeds and faeces.

(3) Percentages of protein, phosphorus and calcium derived by applying the regression equations to the percentages of these constituents in organic-matter faeces.

These equations are:—

$$y = 3{\cdot}34\ (x - 5{\cdot}5)^{0{\cdot}60}$$

where $y =$ *per cent.* protein in dry-matter pasture.

$x =$ *per cent.* protein in organic-matter faeces.

$$y = 0{\cdot}057 + 0{\cdot}364\ x$$

where $y =$ *per cent.* P in dry-matter pasture.

$x =$ *per cent.* P in organic-matter faeces.

$$y = 0{\cdot}019 + 0{\cdot}405\ x$$

where $y =$ *per cent.* Ca in a diet.

$x =$ *per cent.* Ca in organic-matter faeces.

IV. DISCUSSION

In Herd 1, percentages of phosphorus and calcium derived from regression are of the order of magnitude of the percentages determined by analysis of the feed. Protein estimated from regression is considerably less than protein from analysis of the feed.

In Herd 2, percentages of protein, phosphorus and calcium in the whole diet must lie between the levels in the two feeds comprising the diet. Phosphorus and calcium derived from regression both lie about midway between the percentages in the two feeds. Protein derived from regression is considerably less than protein in the two feeds.

For Herd 3, if the two components of the diet, forest grass and irrigated clover, are eaten in the proportion of 2 to 1, then the mixed diet contains 16·3 per cent. protein, 0·23 per cent. phosphorus and 0·55 per cent. calcium. This ratio gives satisfactory agreement with the regression estimates for phosphorus and calcium but the estimate for protein is low and obviously incorrect.

TABLE 1

Feeding Practices, Analysed Percentages of Protein, Phosphorus and Calcium in Feeds on a Dry-matter Basis and in Faeces on an Organic-matter Basis, and Percentages of these Constituents derived from Regression

Herd No.	Feeding Practice	Analyses of Feeds and Faeces				Regression Estimates		
		Feeds and Faeces	Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)
1	Grazing irrigated pasture only	Faeces	18·2	0·96	2·54	15·2	0·41	1·05
		Irrigated pasture	26·8	0·43	1·27	..	..	..
2	Grazing irrigated pasture and lucerne only	Faeces	21·1	1·01	4·16	17·2	0·42	1·70
		Irrigated pasture	30·1	0·47	1·92	..	..	..
		Lucerne ..	28·8	0·37	1·53	..	..	..
3	Grazing irrigated clover and native forest grass	Faeces	13·7	0·45	1·38	11·7	0·22	0·58
		Irrigated clover	30·6	0·37	1·24	..	..	..
		Forest grass ..	9·2	0·16	0·21	..	..	..
4	Grazing irrigated clover and native forest grass	Faeces	17·1	0·49	1·62	14·4	0·24	0·68
		Irrigated clover	27·1	0·28	1·23	..	..	..
		Forest grass ..	9·2	0·16	0·16	..	..	..
5	Grazing irrigated pasture and paspalum	Faeces	14·3	0·65	1·48	12·2	0·29	0·61
		Irrigated pasture	23·6	0·48	0·84	..	..	..
		Paspalum ..	11·2	0·26	0·46	..	..	..
6	Grazing irrigated clover and paspalum	Faeces	18·6	0·75	1·80	15·5	0·33	0·75
		Irrigated clover	27·1	0·38	1·33	..	..	..
		Paspalum ..	13·7	0·32	0·40	..	..	..
7	Grazing lucerne, oats and paspalum	Faeces	14·6	0·69	2·42	12·4	0·31	1·00
		Lucerne ..	25·2	0·38	1·99	..	..	..
		Oats	16·4	0·73	0·48	..	..	..
		Paspalum ..	12·2	0·25	0·29	..	..	..
8	Grazing kikuyu grass and supplemented with 5 lb concentrate	Faeces	15·2	1·25	1·76	12·9	0·51	0·73
		Kikuyu grass ..	11·4	0·26	0·88	..	..	..
		Concentrate ..	19·5	1·19	1·09	..	..	..
9	Entirely hand-fed lucerne hay and concentrates	Faeces	19·2	1·16	1·64	15·9	0·48	0·68
		Composite feed sample	17·2	0·47	0·54	..	..	..
10	Grazing forest grass and paspalum supplemented with 5 lb (dry matter) of maize and concentrate	Faeces	14·2	0·74	1·33	12·1	0·33	0·56
		Grass	10·0	0·26	0·32	..	..	..
		Supplement ..	14·7	0·47	0·72	..	..	..
11	Entirely hand-fed lucerne hay and concentrates, supplemented with bonemeal	Faeces	22·2	..	..	17·9	..	..
		Composite feed sample	19·4	..	..	..	..	..

For Herd 4, if the ratio of forest grass to irrigated clover is 1 to 1, then the diet contains 18·1 per cent. protein, 0·22 per cent. phosphorus and 0·69 per cent. calcium. As in Herd 3 there is satisfactory agreement with the regression estimates of phosphorus and calcium but the estimate for protein is low.

For Herd 5, if the ratio of paspalum grass to irrigated clover is 2 to 1, then the diet contains 15·3 per cent. protein, 0·33 per cent. phosphorus and 0·59 per cent. calcium. The levels of phosphorus and calcium are of the same order of magnitude as the estimates from regression. The regression estimate for protein is low.

For Herd 6, if the ratio of paspalum grass to irrigated clover is 2 to 1, then the diet contains 18·1 per cent. protein, 0·34 per cent. phosphorus and 0·71 per cent. calcium. This ratio gives satisfactory agreement with the regression estimates for phosphorus and calcium but the estimate for protein is low.

In Herd 7 the regression estimate of 1·00 per cent. calcium suggests a large amount of lucerne in the diet, as this is the only constituent of the diet of very high calcium content. The regression estimate for protein is obviously much less than the actual protein content of the diet.

In Herd 8, if a 30 lb dry-matter intake is assumed, the ratio of grass to concentrate in the diet is 5 to 1. Thus the diet would contain 12·7 per cent. protein, 0·41 per cent. phosphorus and 0·91 per cent. calcium. These levels are of the same order of magnitude as the estimates from regression.

In Herd 9, the difference between protein derived from regression and protein determined by analysis of the feed is less than the standard error of estimate of 1·4 per cent. protein in the regression of protein in pasture and in faeces.

In Herd 10, a dry-matter intake of 30 lb would mean a ratio of 5 parts pasture to 1 part concentrate. Thus the diet would contain 10·8 per cent. protein, 0·30 per cent. phosphorus and 0·39 per cent. calcium. At the time of sampling the season was a particularly dry one and milk production had fallen to a low level. A low feed intake was highly probable. At an intake of 15 lb dry matter the ratio is 2 to 1 and the diet would contain 11·6 per cent. protein, 0·33 per cent. phosphorus and 0·46 per cent. calcium. These levels approximate more closely to the estimates from regression.

In Herd 11 the difference between protein determined by analyses of the feed and protein derived from regression is just outside the standard error of estimate of 1·4 per cent. protein.

It is evident that at high protein levels in diets of irrigated pasture, irrigated clover and lucerne, the regressions markedly under-estimate the protein content

of the diet. From published digestibility data, the protein content of faeces derived from lucerne is much less than that derived from grass of the same protein content. Thus an under-estimate would be expected. However, for clover and grass of high protein content the lower estimate from regression can be explained only in terms of a higher apparent protein digestibility and/or a lower dry-matter digestibility than those shown in the literature for similar feeds.

V. CONCLUSIONS

The regressions for phosphorus and calcium developed on data from relatively uniform diets may be used to estimate the percentages of these elements in diets comprising feeds differing widely in chemical composition.

The percentages of phosphorus and calcium in feeds and faeces may be used to estimate the approximate proportions of two feeds comprising a diet. A limitation is that these feeds must have sufficiently wide differences in phosphorus or calcium content. From this proportion the protein content in a mixed diet may be calculated when the protein content of each feed in the diet is known. Reasonable deductions may be made also with regard to the total dry-matter intake when a supplement of known composition is fed in known amounts.

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NUTRITION OF GRAZING CATTLE

4. Selectivity Exhibited by Grazing Cattle

By K. W. MOIR, B.Sc.*

SUMMARY

A comparison was made between the percentages of protein, phosphorus and calcium in total available pasture and the percentage of these constituents in the pasture representative of that selected by grazing cattle.

The two pastures examined were an *Axonopus affinis* and a *Paspalum dilatatum* dominant pasture. For both pastures the selected pasture contained about 50 per cent. more protein and about 30 per cent. more phosphorus than the total available pasture.

Calcium levels in the selected pasture were comparable with those in the total available pasture.

I. INTRODUCTION

Plant and animal factors involved in selective grazing have been discussed by Hardison, Reid, Martin, and Woolfolk (1954). They compared digestibility data derived from grazing steers with digestibility data from steers hand-fed forage clipped from areas adjacent to those allotted to grazing. They found that the average diet selected by the grazing steers contained 23·3 per cent. more crude protein, 37·3 per cent. more fat, 25·6 per cent. more ash and 16·8 per cent. less crude fibre than the whole herbage.

Regressions relating faecal and pasture composition (Moir 1960*a*, 1960*b*) permit estimates of the protein, phosphorus and calcium contents of pastures selectively grazed. It is the object of the investigations reported in this paper to examine the degree of selectivity exhibited by cattle grazing (1) *Axonopus affinis* (narrow-leaf carpet grass) dominant pasture, and (2) *Paspalum dilatatum* (paspalum) dominant pasture.

II. METHODS

Faecal samples were taken as described by Moir (1960*a*).

The pasture sampling technique described by Moir (1960*a*) was used to obtain samples representative of pasture selectively grazed.

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Quadrat sampling was used to obtain samples representative of the total available pasture. Six random quadrats each of 4 sq. ft. were cut to 2 in. above ground level and the total pasture collected in this manner was sub-sampled for analysis.

Methods for the determination of protein, phosphorus and calcium were those described by Moir (1960*a*, 1960*b*).

III. RESULTS AND DISCUSSION

The chemical composition of pasture selected by eight Hereford heifers grazing a 4 ac paddock of carpet grass dominant pasture is compared in Table 1 with the chemical composition of the total pasture.

TABLE 1

Comparison of Chemical Composition of Selected Diets of Carpet Grass Dominant Pasture with Chemical Composition of Total Pasture

Month of Sampling	Chemical Composition of Selected Diet (Dry Matter)						Chemical Composition of Total Pasture		
	From Faecal Analysis			From Pasture Analysis			From Pasture Analysis		
	Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)
May ..	9·8	0·20	0·21	9·8	0·23	0·19	6·7	0·15	0·16
June ..	9·6	0·20	0·21	9·6	0·22	0·24	6·3	0·15	0·34
July ..	10·5	0·20	0·25	8·2	0·22	0·27	5·7	0·15	0·29
August ..	9·8	0·19	0·23	7·9	0·17	0·21	5·8	0·14	0·31
Mean ..	9·9	0·20	0·23	8·9	0·21	0·23	6·1	0·15	0·28

The chemical composition of the pasture selected by six cows of mixed breed grazing a 6 ac paddock of paspalum dominant pasture is compared in Table 2 with the chemical composition of the total pasture.

TABLE 2

Comparison of Chemical Composition of Selected Diets of Paspalum Dominant Pasture with Chemical Composition of Total Pasture

Date of Sampling	Chemical Composition of Selected Diet (Dry Matter)						Chemical Composition of Total Pasture		
	From Faecal Analysis			From Pasture Analysis			From Pasture Analysis		
	Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)
May 14 ..	12·8	0·34	0·38	13·0	0·29	0·30	8·7	0·23	0·21
May 28 ..	11·4	0·31	0·39	10·7	0·29	0·34	8·1	0·26	0·29
June 10 ..	11·9	0·27	0·37	11·1	0·29	0·30	8·2	0·28	0·35
June 24 ..	12·3	0·26	0·35	12·0	0·27	0·22	7·4	0·15	0·34
July 9 ..	11·5	0·24	0·38	10·2	0·24	0·43	7·3	0·18	0·34
Mean ..	12·0	0·28	0·37	11·4	0·28	0·32	7·9	0·22	0·31

In Table 2 there is satisfactory agreement between the analysed values of protein, phosphorus and calcium in the grasses sampled by a technique designed to yield samples representative of grasses grazed and the values of these constituents determined from the appropriate regressions relating pasture and faecal composition. Similarly, in Table 1 there is satisfactory agreement in the May and June samples. The discrepancy in the values recorded for the July and August samples is considered to be due mainly to pasture sampling error. In this period from July to August most of the pasture was very short and there was some uncertainty whether the grasses sampled had been recently grazed.

When the means of the results of the analyses of pasture samples representative of grasses grazed and the results derived from regression are considered, the selected pasture in both experiments contained about 50 per cent. more protein and about 30 per cent. more phosphorus than the total available pasture sampled by quadrats. Calcium levels in the selected pasture were comparable with those in the total available pasture.

On both pastures, short green grass was available at all times and this was preferentially grazed. Although some of the mature grasses were grazed, it does not appear to be likely from the observations made that the reduction of total available carpet grass pasture in July and August was due entirely to grazing. There would have been considerable losses due to trampling with the heavy rate of stocking of two beasts to the acre for this type of pasture. Weathering would also have contributed to the loss of the dry and brittle mature grasses.

These findings stress the inadequacy of analyses of samples of total available pasture in relation to studies on grazing cattle in Queensland.

IV. ACKNOWLEDGEMENTS

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NUTRITION OF GRAZING CATTLE

5. A Survey of the Nutrition of Dairy Cattle in South-eastern Queensland

By K. W. MOIR, B.Sc.*

SUMMARY

A survey was made of the nutrition of dairy cattle on 68 farms in south-eastern Queensland.

The protein, phosphorus and calcium levels in selectively grazed pasture were related to the class of country, the overall appearance of pastures and the predominant pasture species grazed.

On only two farms was the concentration of protein and phosphorus in selectively grazed pasture below the maintenance requirements of cattle. On other properties a deficiency of protein, phosphorus or calcium was related to a low intake of pasture and thus an associated deficiency of other nutrients.

The decline in milk production in winter was due primarily to low feed intakes.

Total production of milk was related to initial production. Inadequate nutrition of dry cows was a major cause of low total production.

I. INTRODUCTION

In south-eastern Queensland, the growing season for the main pasture species extends from the time of the early summer rains, usually in November, through the hot, wet months of December and January when pasture growth rate is maximum, until late February, when the grasses which have not been heavily grazed reach maturity. During winter, there is often little effective rainfall, and varying degrees of drought are encountered in the late winter months. To obtain winter production, cattle on unimproved pasture must be fed a supplement from late autumn until the pasture comes away following the early summer rains. Supplementary feeding is provided by fodder conservation, the growing of winter crops, the use of irrigated pasture or the purchase of concentrates and hays. The efficient utilization of the summer pasture and the winter feeds requires a knowledge of the nutritional factors which limit production in the different seasons.

The purpose of this survey was to determine the major nutritional factors limiting production of dairy cattle in south-eastern Queensland. Farms were classified on the basis of class of country to determine whether recommendations could be made which would overcome nutritional problems within each classification.

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II. MATERIALS AND METHODS

(a) Area Investigated

The area investigated extended 60 miles north and south and 40 miles west of Brisbane. Sixty-eight properties were included in the survey. The area was surveyed from February 1956 to February 1957.

(b) Seasonal Conditions

Seasonal conditions during the summer in early 1956 were good in the area, except in some districts which missed much of the summer rainfall. Heavy cyclonic rains fell throughout the area in March and April and provided a good carry-over of feed and a good green "pick" throughout most of the winter. From April, no effective rainfall was recorded and by November some districts were affected by drought. Late and only moderate rainfall in the following summer preceded a severe drought in 1957.

(c) Classification of Farms

Farms were classified according to the predominant class of country on which they were situated. The class of country is connoted by original vegetation, soil type and predominant pasture species. These classes were:

(1) *Rain-forest*

(*a*) Heavy rain-forest, occurring where annual rainfall is 45–80 in.; better soils such as the deep red loams of moderate fertility and good structure; after clearing the introduced grasses kikuyu grass (*Pennisetum clandestinum*) and paspalum (*Paspalum dilatatum*) predominant, usually associated with Rhodes grass (*Chloris gayana*) on the slopes.

(*b*) Medium rain-forest on black soils; after clearing, paspalum and Rhodes grass predominant.

(*c*) Light rain-forest on shallow soils; after clearing, self-established blue couch grass (*Digitaria didactyla*) predominant, with some paspalum and native forest grasses such as wire grasses (species of *Aristida*).

(*d*) Marginal rain-forest, usually fringing heavy rain-forest on plateau edges; some eucalypts; kikuyu grass and paspalum degenerating into carpet grass (*Axonopus affinis*).

(2) *Open Forest*

(*a*) Heavy soils merging into the black soils of medium rain-forest; forest blue grass (*Bothriochloa intermedia*) and pitted blue grass (*Bothriochloa decipiens*) predominant, associated with paspalum and Rhodes grass.

(*b*) Light and heavy soils of blue-gum and apple-tree flats; paspalum and blue couch grass predominant; wire grasses on the ridges.

(*c*) Podsolized and sandy ridge soils or stony shallow ridges; poor forest grasses, such as wire grasses, kangaroo grass (*Themeda australis*) and bunch spear grass (*Heteropogon contortus*), predominant.

(*d*) Podsolized and sandy ridge soils or stony shallow ridges associated with heavy soils merging into the black soils of medium rain-forest.

(*e*) Podsolized and sandy ridge soils or stony shallow ridges associated with light and heavy soils; paspalum degenerating into carpet grass on the flats.

(*f*) Wallum-type country; eucalypts and tea-trees; wire grasses, kangaroo grass and bunch spear grass predominant.

(3) *Eucalypt Forest/Bastard Scrub*

High-rainfall areas; podsolized soils; paspalum sown after clearing degenerating into carpet grass.

(4) *Lowlands*

Soils with high water-table in summer and restricted drainage; swamp oak and some tea-tree; blue couch, green couch (*Cynodon dactylon*), water couch (*Paspalum distichum*) and carpet grass predominant.

(d) Classification on Overall Appearance of Pasture

The overall appearance of the pasture on each farm was described as:

(1) *Lush green.*—A uniform lush green sward.

(2) *Green.*—An overall appearance of greenness but some drier areas evident.

(3) *Green to dry.*—The overall appearance not easily defined as either green or dry. Thus, there may be an overall tinge of green or scattered areas of green and dry pastures.

(4) *Dry.*—An overall appearance of dryness, but green growth evident at the base of mature grasses when inspected more closely.

(5) *Very dry.*—Little or no evidence of green growth on close inspection.

(e) Sampling

The procedures used for sampling pasture and faeces were those described by Moir (1960*a*).

(f) Analysis

The methods used for determining protein, phosphorus, calcium and ash were those described by Moir (1960*a*, 1960*b*).

(g) Estimation of Chemical Composition in Selectively Grazed Pasture

For herds in which there were cows not supplemented, the regressions of Moir (1960*a*, 1960*b*) were used to relate protein, phosphorus and calcium in faeces to the percentages of these constituents in the selectively grazed pasture.

For herds in which all cows were supplemented, protein, phosphorus and calcium were determined from analysis of pasture samples only.

(h) Estimation of Chemical Composition in the Diets of Supplemented Herds

The regressions relating faecal and pasture phosphorus and calcium, shown by Moir (1960*c*) to be applicable to mixed diets, were used to estimate phosphorus and calcium. Protein was estimated by the methods of Moir (1960*c*), which were based on the calculated approximate proportion of each feed in the diet and the estimated protein level in each feed, and on adjustments to the regression relating faecal and pasture protein.

(i) Milk Production Records

Milk production records were obtained where possible from the Herd Recording Section of the Queensland Department of Agriculture and Stock. The average milk production of cows lactating for 2, 4 and 6 months in each herd at the time of sampling was calculated. Cows which had dried off before 6 months were not included in calculating these averages.

In herds which were not regularly recorded, the farmers' estimates of the production of cows in the middle of their lactations were taken.

III. RESULTS

A summary of survey results of farms in each classification is given in Tables 1–11. The data recorded on each farm are:

(1) Locality of the farm.

(2) Date of sampling.

(3) Overall appearance of the pasture at the time of sampling.

(4) Predominant pasture species grazed at the time of sampling.

(5) Supplements used at the time of sampling.

TABLE 1

Summary of Survey Results of Farms on Heavy Rain-Forest, Red Soils. (Classification 1a)

Herd No.	Locality	Month of Sampling	Overall Appearance of Pasture	Predominant Grasses Grazed	Supplementary Feeds	Chemical Composition of Pasture (Dry matter)			Chemical Composition of Diet (Dry matter)			Milk Yield (lb) for Lactation Period (months)		
						Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)	2	4	6
1	Tamborine Mountain	Apr., 1956	Lush green ..	Kikuyu ..	Nil	18·0	0·43	0·36	..	..	..		30	
		Feb., 1957	Green ..	Kikuyu ..	Nil	13·9	0·31	0·27	..	..	..		25	
		Oct., 1956	Green to dry	Kikuyu ..	Nil	12·9	0·25	0·35	..	..	..		20	
2	Maleny ..	Apr., 1956	Green to lush	Kikuyu ..	2 lb	15·3	0·38	0·27	..	..	..	20	16	11
		Jan., 1957	Green ..	Kikuyu ..	Meat-and-bone-meal	14·0	0·29	0·34	..	..	..	24	21	19
		Nov., 1956	Green to dry	Paspalum ..	to the highest	12·0	0·26	0·30	..	..	..	24	18	15
		Oct., 1956	Green to dry	Paspalum ..	producing cows	12·0	0·23	0·37	..	..	..	21	16	14
		Aug., 1956	Green to dry	Paspalum ..	in all months	11·5	0·28	0·45	..	..	..	19	16	10
3	Beechmont ..	Jan., 1957	Green ..	Paspalum ..	Nil	14·8	0·27	0·34	..	..	..	23	21	18
4	Maleny ..	Jan., 1957	Green ..	Paspalum ..	Nil	14·4	0·29	0·46	..	..	..	18	15	11
		Sept., 1956	Green to dry	Paspalum and carpet	Nil	11·7	0·20	0·42	..	..	..	15	12	10
5	Maleny ..	Jan., 1957	Green ..	Kikuyu ..	Nil	14·1	0·36	0·30	..	..	..	25	24	22
		Sept., 1956	Green to dry	Paspalum ..	5 lb conc. to top cows	11·4	0·26	0·88	13·0	0·51	0·73	26	21	17
6	Beechmont ..	Jan., 1957	Green ..	Kikuyu ..	Nil	13·4	0·38	0·28	..	..	..		15	

(6) Percentages of protein, phosphorus and calcium in selectively grazed pasture.

(7) Percentages of protein, phosphorus and calcium in the diets of supplemented herds.

(8) Average milk yields of cows lactating for 2, 4 and 6 months at the time of sampling in recorded herds and estimates of production of cows at about 4 months in lactation for non-recorded herds.

In Table 12 are recorded the percentages of protein, phosphorus and calcium in selectively grazed pasture on podsolized and sandy ridge soils or stony shallow ridges (Classification 2c) used widely for dry cows and heifers.

Table 13 is assembled from data in Tables 1–12. This table records the means and the maximum variation from the means of protein, phosphorus and calcium contents of selectively grazed pasture when there are two or more analyses of pastures with similar attributes in each classification of class of country.

In Table 14 are shown minimum percentages of protein required in pasture for maintenance plus milk production. Using the relationship between apparent protein digestibility and protein percentage in pasture calculated by Moir (1960*a*), these minimum percentages are calculated from the amounts of digestible protein recommended by the National Research Council (1956) for maintenance and production of a 1,000 lb cow. These percentages have been calculated for dry-matter intakes ranging from 15 to 30 lb daily. The minimum percentages of protein required in mixed diets are dependent upon the apparent protein digestibilities for each feed comprising a diet. The general relationship of apparent protein digestibility and protein percentage in different classes of feedstuffs shown by Glover, Duthie, and French (1957) indicates that the data in Table 14 may be used generally with little error for mixed diets, particularly when roughage constitutes the principal part of a diet.

In Tables 15 and 16 are minimum percentages of phosphorus and calcium in a diet required for maintenance plus milk production at dry-matter intakes of 15–30 lb. These percentages are calculated from the amounts of phosphorus and calcium for maintenance and milk production recommended for a 1,000 lb cow by the National Research Council (1956).

Although some of the dry-matter intakes given in Tables 14, 15 and 16 may be rarely encountered, they are included to emphasize the concentrations of protein, phosphorus and calcium which would be required for an extreme range of conditions.

TABLE 2

Summary of Survey Results of Farms on Medium Rain-Forest, Black Soils (Classification 1b)

Herd No.	Locality	Month of Sampling	Overall Appearance of Pasture	Predominant Grasses Grazed	Supplementary Feeds	Chemical Composition of Pasture (Dry matter)			Chemical Composition of Diet (Dry matter)			Milk Yield (lb) for Lactation Period (months)		
						Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)	2	4	6
1	Gleneagle ..	Jan., 1957	Green to lush	Paspalum ..	Nil	15·4	0·30	0·34	..	..	..	21	19	17
		June, 1956	Green ..	Paspalum ..	Irrigated white clover	13·7	0·32	0·40	18·0	0·33	0·75	22	18	15
2	Veresdale ..	Jan., 1957	Green ..	Paspalum ..	Nil	14·1	0·29	0·38	..	..	..	22	20	15
		Feb., 1956	Green ..	Paspalum and Rhodes	Nil	13·4	0·30	0·28	..	..	..	19	18	15
		Sept., 1956	Green to dry	Paspalum ..	3 lb concentrates, roughage and bonemeal to top cows	11·1	0·27	0·47	13·0	0·39	0·60	19	17	10
3	Veresdale ..	Jan., 1957	Green ..	Paspalum and Rhodes	Nil	12·7	0·33	0·38	..	..	..	25	21	17
		Apr., 1956	Green ..	Paspalum and Rhodes	Nil	12·2	0·31	0·30	..	..	..	19	17	12
		Feb., 1957	Green to dry	Paspalum and Rhodes	Nil	9·8	0·22	0·28	..	..	..	25	22	17
		Mar., 1956	Green to dry	Paspalum and Rhodes	Nil	9·6	0·23	0·35	..	..	..	23	19	15
		Nov., 1956	Dry	Paspalum and Rhodes	Silage and meatmeal	..	..	..	13·0	0·27	0·68	21	16	12
4	Lamington ..	June, 1956	Green to dry	Paspalum ..	Nil	11·4	0·27	0·38	..	..	..		15	

TABLE 3

Summary of Survey Results of Farms on Light Rain-Forest, Shallow Soils (Classification 1c)

Herd No.	Locality	Month of Sampling	Overall Appearance of Pasture	Predominant Grasses Grazed	Supplementary Feeds	Chemical Composition of Pasture (Dry matter)			Chemical Composition of Diet (Dry matter)			Milk Yield (lb) for Lactation Period (months)		
						Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)	2	4	6
1	Whiteside ..	Jan., 1957	Green ..	Blue couch ..	Grazing panicum and irrigated pasture	11·9	0·26	0·33	12·0	0·31	0·44	20	17	16
		Mar., 1956	Green ..	Blue couch ..	Nil	11·4	0·28	0·31	..	..	..	18	16	14
2	Lacey's Creek	Apr., 1956	Green ..	Blue couch ..	Nil	10·7	0·24	0·35	..	..	..	22	19	15
3	Narangba ..	Jan., 1957	Green ..	Blue couch and carpet	Nil	10·3	0·20	0·28	..	..	..		15	
4	Bald Hills ..	June, 1956	Green to dry	Blue couch ..	Entirely hand-feeding	..	..	..	19·0	..	..	30	24	18
5	Upper Caboolture	Dec., 1956	Green to dry	Blue couch and forest grass	3 lb concentrates and bonemeal	9·0	0·23	0·51	10·0	0·30	0·70	16	11	8
6	Kilcoy ..	Feb., 1957	Green to dry	Blue couch and forest grass	Nil	8·9	0·21	0·34	..	..	..		15	
7	Moggill ..	Dec., 1956	Green ..	Blue couch ..	Up to 10 lb concentrates; grazing crops	..	..	..	14·0	0·53	1·15	48	33	29
		Feb., 1957	Green ..	Blue couch ..		..	..	..	13·0	0·44	0·51	44	36	24
8	Highvale ..	Sept., 1956	Dry	Blue couch ..	5 lb concentrates and roughage	..	..	..	12·5	0·35	0·60	18	15	12
9	Upper Caboolture	Oct., 1956	Dry	Blue couch ..	Irrigated pasture — little outside grazing	..	..	..	18·0	0·46	0·68	17	16	13

TABLE 4

Summary of Survey Results of Farms on Marginal Rain-Forest on Plateau Edges (Classification 1d)

Herd No.	Locality	Month of Sampling	Overall Appearance of Pasture	Predominant Grasses Grazed	Supplementary Feeds	Chemical Composition of Pasture (Dry matter)			Chemical Composition of Diet (Dry matter)			Milk Yield (lb) for Lactation Period (months)		
						Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)	2	4	6
1	Reesville ..	Jan., 1957	Green ..	Carpet and kikuyu	Nil	13·2	0·25	0·28	..	..	..		12	
		Oct., 1956	Green to dry	Carpet and kikuyu	Nil	10·3	0·19	0·30	..	..	..		10	
2	Mount Mee ..	Feb., 1957	Green to dry	Carpet ..	Nil	9·0	0·16	0·25	..	..	..		8	
3	Witta ..	Jan., 1959	Green to dry	Carpet ..	Nil	8·5	0·17	0·29	..	..	..		8	

TABLE 5

Summary of Survey Results of Farms on Open Forest, Heavy Soils (Classification 2a)

Herd No.	Locality	Month of Sampling	Overall Appearance of Pasture	Predominant Grasses Grazed	Supplementary Feeds	Chemical Composition of Pasture (Dry matter)			Chemical Composition of Diet (Dry matter)			Milk Yield (lb) for Lactation Period (months)		
						Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)	2	4	6
1	Veresdale ..	Jan., 1957	Green to dry	Forest grass and paspalum	Improved pasture	12·3	0·30	0·34	14·0	0·29	0·51	24	22	19
		Feb., 1957	Green to dry	Forest grass and paspalum	Lucerne and Sudan grass	..	..	..	11·0	0·20	0·47	25	21	17
		Nov., 1956	Dry	Forest grass ..	Silage and meatmeal	..	..	..	12·0	0·29	0·71	20	16	15
2	Gleneagle ..	Jan., 1957	Green to dry	Forest grass and paspalum	Improved pasture	11·6	0·26	0·29	15·0	0·34	0·54	32	25	15
		Mar., 1956	Green to dry	Forest grass and paspalum	Irrigated pasture	11·6	0·34	0·39	14·0	0·33	0·45	25	23	20
3	Jimboomba ..	Jan., 1957	Green to dry	Forest grass and paspalum	Improved pasture and chaffed maize	11·0	0·30	0·28	12·0	0·31	0·38		17	
		Apr., 1956	Green to dry	Forest grass and paspalum	Chaffed maize	..	..	..	7·5	0·23	0·29		12	
4	Woolmar ..	Jan., 1957	Green to dry	Rhodes and forest grass	2 lb corn-and-cob to top cows	10·6	0·30	0·34	..	..	..		17	
		Oct., 1956	Dry	Rhodes and forest grass	5 lb corn-and-cob to top cows	7·7	0·25	0·37	..	..	..		12	
5	Veresdale ..	Jan., 1957	Green to dry	Forest grass ..	Chaffed maize and lucerne hay	10·0	0·26	0·32	12·0	0·33	0·56		17	
		Apr., 1956	Dry	Forest grass ..	Chaffed maize	..	..	..	7·6	0·27	0·36		12	

TABLE 6

Summary of Survey Results of Farms on Open Forest, Light and Heavy Soils (Classification 2b)

Herd No.	Locality	Month of Sampling	Overall Appearance of Pasture	Predominant Grasses Grazed	Supplementary Feeds	Chemical Composition of Pasture (Dry matter)			Chemical Composition of Diet (Dry matter)			Milk Yield (lb) for Lactation Period (months)		
						Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)	2	4	6
1	Yatala ..	Jan., 1957	Green ..	Paspalum ..	2 lb grain to top cows	14·4	0·30	0·27	..	..	..	18	16	16
		July, 1956	Green to dry	Paspalum ..	Irrigated clover	..	..	..	16·0	0·36	0·68	17	14	11
2	Yatala ..	Jan., 1957	Green ..	Paspalum and blue couch	Nil	13·1	0·25	0·23	..	..	..		15	
		July, 1956	Dry	Paspalum and blue couch	3 lb concentrates to top cows	9·1	0·25	0·26	10·0	0·23	0·36		12	
3	Amberley ..	Feb., 1957	Green ..	Paspalum and blue couch	3 lb lucerne hay	12·1	0·28	0·28	13·0	0·26	0·41	18	15	11
4	Waterford ..	Feb., 1956	Green ..	Paspalum and blue couch	Nil	12·0	0·39	0·26	..	..	..	14	12	10
		Apr., 1956	Green ..	Paspalum and blue couch	Nil	11·9	0·30	0·30	..	..	..	14	10	9
5	Amberley ..	Feb., 1957	Green ..	Paspalum and blue couch	Nil	12·0	0·27	0·53	..	..	..		12	
6	Waterford ..	Apr., 1956	Green ..	Paspalum and blue couch	Nil	11·7	0·32	0·29	..	..	..		12	
7	Upper Caboolture	Dec., 1956	Green ..	Paspalum and blue couch	Nil	11·7	0·28	0·33	..	..	..		15	
8	Upper Caboolture	Dec., 1956	Green ..	Paspalum and blue couch	Irrigated pasture	11·7	0·24	0·36	13·0	0·30	0·47	22	16	14
9	Amberley ..	Feb., 1957	Green to dry	Paspalum ..	2 lb concentrates and bonemeal	10·4	0·33	0·31	12·0	0·39	0·60		15	

TABLE 7

Summary of Survey Results of Farms on Open Forest, Podsolized and Sandy Ridge Soils or Stony Shallow Ridges Associated with Heavy Soils Merging into the Black Soils of Medium Rain-Forest (Classification 2d)

Herd No.	Locality	Month of Sampling	Overall Appearance of Pasture	Predominant Grasses Grazed	Supplementary Feeds	Chemical Composition of Pasture (Dry matter)			Chemical Composition of Diet (Dry matter)			Milk Yield (lb) for Lactation Period (months)		
						Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)	2	4	6
1	Veresdale ..	Jan., 1957	Green to dry	Paspalum ..	Nil	12·9	0·23	0·28	..	..	..		15	
		Oct., 1956	Dry	Paspalum and forest grass	3 lb meal to top cows	10·7	0·17	0·24	..	..	..		12	
2	Rosevale ..	Dec., 1956	Green to dry	Paspalum ..	Irrigated pasture	12·3	0·26	0·36	14·0	0·29	0·62	24	19	16
3	Mount Beppo	Feb., 1957	Green to dry	Paspalum and forest grass	Irrigated pasture	10·5	0·20	0·21	13·0	0·27	0·48	25	22	19
		Sept., 1956	Dry	Paspalum and forest grass	Irrigated pasture	9·2	0·16	0·16	16·0	0·22	0·58	28	23	22
4	Kilcoy ..	Nov., 1956	Green to dry	Rhodes following burning	Nil	8·9	0·22	0·25	..	..	..		15	
5	Mount Beppo	Sept., 1956	Dry	Paspalum and forest grass	Nil	9·0	0·17	0·37	..	..	..		7	

TABLE 8

Summary of Survey Results of Farms on Open Forest, Podsolized and Sandy Ridge Soils or Stony Shallow Ridges Merging into Light and Heavy Soils (Classification 2e)

Herd No.	Locality	Month of Sampling	Overall Appearance of Pasture	Predominant Grasses Grazed	Supplementary Feeds	Chemical Composition of Pasture (Dry matter)			Chemical Composition of Diet (Dry matter)			Milk Yield (lb) for Lactation Period (months)		
						Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)	2	4	6
1	Tamborine Village	Feb., 1957	Green to dry	Paspalum and carpet	Nil	9·9	0·21	0·24	..	..	..		12	
2	Tamborine Village	Apr., 1956	Green to dry	Paspalum and carpet	Nil	10·3	0·20	0·26	..	..	..		12	

TABLE 9

Summary of Survey Results of Farms on Wallum Type Country (Classification 2f)

Herd No.	Locality	Month of Sampling	Overall Appearance of Pasture	Predominant Grasses Grazed	Supplementary Feeds	Chemical Composition of Pasture (Dry matter)			Chemical Composition of Diet (Dry matter)			Milk Yield (lb) for Lactation Period (months)		
						Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)	2	4	6
1	Elimbah ..	Jan., 1957	Dry	Forest grass ..	Improved pasture	6·1	0·08	0·13	9·0	0·76	0·26		<5	
		July, 1956	Very dry ..	Forest grass ..	Nil	5·7	0·07	0·10	..	..	..		<5	
2	Deception Bay	Sept., 1956	Very dry ..	Forest grass ..	Elephant grass	5·4	0·05	0·10	8·0	0·13	0·19		<5	

TABLE 10

Summary of Survey Results of Farms on Eucalypt Forest/Bastard Scrub Country (Classification 3)

Herd No.	Locality	Month of Sampling	Overall Appearance of Pasture	Predominant Grasses Grazed	Supplementary Feeds	Chemical Composition of Pasture (Dry matter)			Chemical Composition of Diet (Dry matter)			Milk Yield (lb) for Lactation Period (months)		
						Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)	2	4	6
1	Mooloolah ..	Apr., 1956	Green ..	Paspalum ..	4 lb corn-and-cob to some cows	11·8	0·27	0·19	..	..	..		10	
		Nov., 1956	Green to dry	Paspalum and carpet	4 lb meal ..	10·3	0·16	0·19	11·0	0·24	0·22		8	
2	Mooloolah ..	Apr., 1956	Green ..	Paspalum ..	Nil	11·3	0·25	0·28	..	..	..		7	
		Feb., 1957	Green ..	Paspalum and carpet	2 lb meal to some cows	10·6	0·20	0·23	..	..	..		10	
		Nov., 1956	Green to dry	Paspalum and carpet	2 lb meal to some cows	10·2	0·18	0·27	..	..	..		7	
3	Mount Cotton	Dec., 1956	Green to dry	Paspalum and carpet	Nil	9·9	0·15	0·16	..	..	..		10	
		Aug., 1956	Green to dry	Carpet ..	½ lb meatmeal	..	..	..	12·0	0·28	0·43		8	
4	Chevallum ..	Nov., 1956	Green to dry	Carpet ..	Nil	9·7	0·15	0·21	..	..	..		7	
		Feb., 1957	Green to dry	Carpet ..	Nil	8·7	0·16	0·20	..	..	..		8	
5	Buderim ..	Nov., 1956	Green to dry	Carpet ..	Nil	9·3	0·14	0·21	..	..	..		7	
		Feb., 1957	Green to dry	Carpet ..	Nil	8·9	0·14	0·16	..	..	..		8	
6	Mount Cotton	Dec., 1956	Green to dry	Carpet ..	Nil	9·3	0·14	0·22	..	..	..		8	
		Mar., 1956	Green to dry	Carpet ..	Nil	9·2	0·18	0·18	..	..	..		7	
7	Buderim ..	Feb., 1957	Green to dry	Carpet ..	Nil	8·4	0·16	0·16	..	..	..		7	
		Aug., 1956	Green to dry	Carpet ..	2 lb meal to some cows	8·1	0·12	0·19	..	..	..		7	

TABLE 11

Summary of Survey Results of Farms on Lowlands (Classification 4)

Herd No.	Locality	Month of Sampling	Overall Appearance of Pasture	Predominant Grasses Grazed	Supplementary Feeds	Chemical Composition of Pasture (Dry matter)			Chemical Composition of Diet (Dry matter)			Milk Yield (lb) for Lactation Period (months)		
						Protein (%)	P (%)	Ca (%)	Protein (%)	P (%)	Ca (%)	2	4	6
1	Tallebudgera	Oct., 1956	Green to dry	Couch and carpet	Nil	10·7	0·21	0·32	..	..	..	14	10	
2	Mudgeeraba	Nov., 1956	Green to dry	Couch and carpet	Nil	10·6	0·24	0·29	..	..	..	9	10	9
		Jan., 1957	Green to dry	Couch and carpet	Nil	9·5	0·19	0·29	..	..	..	15	12	9
3	Currumbin ..	Jan., 1957	Green to dry	Couch and carpet	Nil	9·6	0·19	0·26	..	..	..	18	15	11
		Aug., 1956	Green to dry	Couch and carpet	5 lb lucerne hay, 2 lb meal	..	..	..	14·0	0·30	0·45	19	16	16
4	Mudgeeraba	July, 1956	Green to dry	Couch and carpet	3 lb meal to some cows	9·6	0·19	0·26	..	..	..	12	11	9
		Nov., 1956	Dry	Couch and carpet	Nil	8·5	0·13	0·26	..	..	..	14	10	9
		Dec., 1956	Dry	Couch and carpet	Bonemeal ..	..	..	..	8·2	0·25	0·53	14	13	11

TABLE 12

Summary of Survey Results of Dry Cow and Heifer Paddocks on Open Forest, Podsolized and Sandy Ridge Soils or Stony Ridges (Classification 2C)

Herd No.	Locality	Month of Sampling	Overall Appearance of Pasture	Predominant Grasses Grazed	Chemical Composition of Pasture (Dry matter)		
					Protein (%)	P (%)	Ca (%)
1	Upper Caboolture	Oct., 1956	Dry	Forest grass, some paspalum	9·3	0·14	0·21
		Jan., 1957	Dry	Forest grass	8·0	0·13	0·28
2	Whiteside	Oct., 1956	Dry	Forest grass, some paspalum	9·3	0·13	0·30
3	Tamborine Village	Oct., 1956	Dry	Forest grass, some paspalum	9·1	0·12	0·27
4	Wonglepong	Sept., 1956	Dry	Forest grass, some paspalum	9·0	0·13	0·26
5	Kilcoy	Feb., 1957	Dry	Forest grass	8·7	0·12	0·17
6	Narangba	Sept., 1956	Dry	Forest grass, some paspalum	8·4	0·13	0·21
7	Benarkin	July, 1956	Dry	Forest grass	7·9	0·13	0·27
8	Moggill	Sept., 1956	Dry	Forest grass following burning	7·5	0·16	0·26
9	Palmview	Sept., 1956	Dry	Forest grass, some carpet	7·5	0·16	0·24
10	Veresdale	Oct., 1956	Dry	Forest grass	7·5	0·15	0·26
11	Veresdale	Nov., 1956	Dry	Forest grass, some Rhodes	7·4	0·19	0·34
12	Highvale	Sept., 1956	Very dry	Forest grass	7·7	0·14	0·36
13	Cedar Grove	Jan., 1957	Very dry	Forest grass	7·4	0·10	0·22
14	Gleneagle	Sept., 1956	Very dry	Forest grass	6·9	0·12	0·23

TABLE 13

Means and Maximum Variations from the Means of Protein, Phosphorus and Calcium in Pasture with Similar Attributes on Each Class of Country

Class of Country	Overall Appearance of Pasture	Predominant Pasture Species Grazed	Mean and Maximum Variations from the Mean					
			Protein (%)		P (%)		Ca (%)	
			Mean	Maximum Variation	Mean	Maximum Variation	Mean	Maximum Variation
1a	Green	Paspalum	14·6	(0·2)	0·28	(0·01)	0·40	(0·06)
	Green	Kikuyu	13·9	(0·5)	0·34	(0·05)	0·30	(0·04)
	Green to dry	Paspalum	11·7	(0·3)	0·26	(0·03)	0·50	(0·38)
1b	Green	Paspalum	13·9	(0·2)	0·31	(0·02)	0·39	(0·01)
	Green	Paspalum and Rhodes	12·8	(0·6)	0·31	(0·02)	0·32	(0·06)
	Green to dry	Paspalum	11·3	(0·2)	0·27	(0)	0·43	(0·05)
	Green to dry	Paspalum and Rhodes	9·7	(0·1)	0·23	(0·01)	0·32	(0·04)
1c	Green	Blue couch grass	11·3	(0·6)	0·26	(0·02)	0·33	(0·02)
	Green to dry	Blue couch and forest grass	9·0	(0·1)	0·22	(0·01)	0·43	(0·09)
1d	Green to dry	Carpet	8·8	(0·3)	0·17	(0·01)	0·27	(0·02)
2a	Green to dry	Forest grass and paspalum	11·6	(0·7)	0·30	(0·04)	0·33	(0·06)
2b	Green	Paspalum and blue couch	12·0	(1·1)	0·29	(0·10)	0·32	(0·21)
2c	Dry	Forest grass and paspalum	9·0	(0·6)	0·13	(0·01)	0·25	(0·05)
	Dry	Forest grass	8·0	(0·7)	0·13	(0·02)	0·25	(0·08)
	Very dry	Forest grass	7·3	(0·4)	0·12	(0·02)	0·27	(0·09)
2d	Green to dry	Paspalum	12·6	(0·3)	0·25	(0·02)	0·32	(0·04)
	Dry	Paspalum and forest grass	9·7	(1·0)	0·17	(0·01)	0·26	(0·11)
2e	Green to dry	Paspalum and carpet grass	10·1	(0·2)	0·21	(0·01)	0·25	(0·01)
2f	Very dry	Forest grass	5·6	(0·2)	0·06	(0·01)	0·10	(0)
3	Green	Paspalum	11·6	(0·3)	0·26	(0·01)	0·24	(0·05)
	Green to dry	Paspalum and carpet	10·1	(0·2)	0·16	(0·02)	0·21	(0·06)
	Green to dry	Carpet	9·0	(0·9)	0·15	(0·03)	0·19	(0·03)
4	Green to dry	Blue couch and carpet	10·0	(0·7)	0·20	(0·04)	0·28	(0·04)

TABLE 14

Minimum Percentages of Protein Required in Pasture for Maintenance and Milk Production of a 1000 lb Cow

Dry-matter Intake (lb)	Minimum Protein Percentage Required in Pasture										
	Milk Production (lb)										
	0	5	10	15	20	25	30	35	40	45	50
15	7·5	9·2	11·0	12·7	14·4	15·8	..	..	..	..	..
20	6·5	7·7	9·0	10·3	11·5	12·7	14·0	..	..	..	..
25	..	7·0	7·7	8·7	9·8	10·8	11·8	12·8	13·8	..	..
30	..	..	7·2	7·9	8·7	9·5	10·3	11·2	12·1	12·9	13·7

TABLE 15

Minimum Percentages of Phosphorus Required in a Diet for Maintenance and Milk Production of a 1000 lb Cow

Dry-matter Intake (lb)	Minimum Phosphorus Percentage Required in a Diet										
	Milk Production (lb)										
	0	5	10	15	20	25	30	35	40	45	50
15	0·12	0·17	0·22	0·27	0·33	0·38	..	..	..	..	..
20	0·09	0·13	0·17	0·21	0·24	0·28	0·32	0·36	..	..	..
25	..	0·10	0·13	0·16	0·20	0·23	0·26	0·29	0·32	..	..
30	..	..	0·11	0·14	0·16	0·19	0·22	0·24	0·27	0·29	0·32

TABLE 16

Minimum Percentages of Calcium Required in a Diet for Maintenance and Milk Production of a 1000 lb Cow

Dry-matter Intake (lb)	Minimum Calcium Percentage Required in a Diet										
	Milk Production (lb)										
	0	5	10	15	20	25	30	35	40	45	50
15	0·12	0·19	0·27	0·34	0·41	0·49	..	..	..	..	..
20	0·09	0·14	0·20	0·26	0·31	0·37	0·42	0·48	..	..	..
25	..	0·12	0·16	0·20	0·25	0·29	0·34	0·38	0·43	..	..
30	..	..	0·13	0·17	0·21	0·24	0·28	0·32	0·36	0·39	0·43

IV. DISCUSSION

From the data in Tables 1 to 11, two major problems of the dairy farms examined are:—

(1.) *The fall in production during winter.*—In spite of supplementary feeding practices, the summer level of production is not maintained from autumn to spring.

(2.) *The low annual productivity in many herds.*—The average annual milk production per cow ranged from less than 200 gal to about 500 gal and the average for the recorded herds was 350 gal in 1956. Herds which were not recorded were in most cases low producing and the average for all herds could be expected to be less than 300 gal.

The survey sample was drawn from a total of 207 herds in nine dairying districts, classified for herd-recording purposes as Beaudesert, Brisbane, Beenleigh, Ipswich, Kilcoy, Landsborough-Caboolture, Maleny, Tamborine Mountain and Beechmont-Currumbin. The herd recording data for these districts (S. E. Pegg, personal communication) indicate that the problems of the dairy farms examined are general in south-eastern Queensland and even in the whole of Queensland's dairying areas.

Three of the districts (Beaudesert, Brisbane and Beenleigh) are on milk supply, which necessitates maximum endeavour to maintain milk production throughout the year. The monthly production averages for 79 herds in these districts for 1956 were:—

—	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Milk (lb) ..	524	494	432	380	382	400	422	454	440	472	438	450
Butterfat (lb) ..	21	21	18	17	17	17	17	17	17	18	17	17

In spite of supplementary feeding practices, a decline from the summer (January and February) level of production is evident. An even greater decline must be anticipated in cream-supply herds, as the economics of cream production are not dependent upon the maintenance of maximum production throughout the year.

The average annual milk production per cow in Moreton Statistical Division, which embraces the nine districts, and in the whole of Queensland for 1949 to 1959 was:—

	Moreton (gal)	Queensland (gal)
1949–50	265	260
1950–51	272	272
1951–52	209	195
1952–53	276	273
1953–54	252	248
1954–55	267	261
1955–56	282	282
1956–57	256	256
1957–58	219	216
1958–59	278	276

The low average productivity in the herds surveyed was therefore typical of herds in Queensland in all years.

Because of the essentially summer rainfall, pasture reaches maturity in late summer with a resultant fall in digestible protein, total digestible nutrients and phosphorus. Pasture regrowth with a resultant rise in food value cannot be expected until the first effective spring or early summer rains. Thus the seasonal decline from the summer level of production in dairy cattle must be largely a nutritional problem. Adverse weather conditions such as cold winds and rain undoubtedly contribute to short-term depressions in milk production.

Productivity of herds in this survey is related to class of country and to supplementary feeding practices. Cows on the poorest classes of country, represented in Tables 4, 8, 9 and 10, produced less than 200 gal of milk annually. On the most fertile classes of country, represented in Tables 1 and 2, the production of cows in some herds receiving little or no supplement was above average. On the intermediate classes of country, a higher rate of supplementation was required to obtain production equivalent to the highest producing herds recorded in Tables 1 and 2. The relationship of productivity to class of country and to the level of supplementary feeding indicates that nutrition must be the most important single factor affecting productivity.

The influence of nutritional factors on productivity must be considered in relation to:—

(1) Summer (January and February) production.

(2) Autumn/spring production.

(3) Total productivity.

(1) *Summer Production.*—The highest producing herds on those classes of country with cows producing at least 2 gal of milk could be expected to consume

25 to 30 lb of dry matter per head daily. At feed intakes of 30 lb, herds in Tables 1 to 11 with cows producing 2 gal of milk in January or February would not be deficient in protein, phosphorus or calcium on the basis of the minimum requirements as set out in Tables 14, 15 and 16. At an intake of 25 lb dry matter, only the highest producing cows in Herd 3, Table 2, could be slightly deficient in protein, phosphorus and calcium. The highest producing cows in Herd 1, Table 5, could be slightly deficient in phosphorus, and some cows in Herd 1, Table 1, could be slightly deficient in calcium.

On the basis of digestibility data of Morrison (1937) and Schneider (1947) for grasses of protein content similar to that encountered in this survey, it could be expected that the total digestible nutrients in the summer pastures would be adequate for the production levels attained. Evidence that a deficiency of protein, phosphorus, calcium or total digestible nutrients was not specifically limiting the summer production in the lower producing herds on each class of country may be obtained by comparing the chemical composition of pasture selected by low and high producing herds. On each class of country, when the same pasture species are grazed and when the protein levels of the selectively grazed pastures are similar, the phosphorus and calcium levels in the selected pastures and the overall appearances of the pastures are also similar (Table 13). It could therefore be expected that under these conditions the percentages of total digestible nutrients in these pastures would be similar. The productivity of the lowest producing pasture-fed herds in summer is therefore not due to lack of total digestible nutrients, protein, phosphorus or calcium in the selected pasture, when there are higher producing herds on no greater bulk of pasture with similar or even lower levels of these nutrients.

Herds on poor classes of country (Tables 4, 8, 9 and 10), with cows producing less than 2 gal of milk, would not be deficient in protein, phosphorus or calcium at dry-matter intakes of 30 lb in the summer months. However, it is unlikely that the dry-matter intakes of low-producing herds would approach 30 lb, and varying degrees of protein, phosphorus or calcium deficiencies could occur at lower feed intake levels. Thus, at dry-matter intakes of 20 lb, a number of herds on the poorest classes of country could have been deficient in protein, phosphorus or calcium. These deficiencies cannot be regarded as specific deficiencies, but rather as deficiencies induced by low feed intakes.

For the area covered in this survey, there was an abundance of pasture in January and February of 1956 and 1957. Thus both field and analytical evidence support the conclusion that low productivity in summer is not a function of quantity or quality of the pasture available at that time.

(2) *Autumn/Spring Production.*—For the higher producing pasture-fed herds in Tables 1 to 11, deficiencies of protein, phosphorus or calcium would not occur from autumn to spring in cows producing 2 gal of milk and consuming 30 lb of dry matter. At an intake of 25 lb, only Herd 3, Table 7, could be

deficient in phosphorus. As stated previously, within any class of country the level of total digestible nutrients in pasture selected by lower producing herds cannot be limiting when there are other herds giving higher production on pasture of similar chemical and botanical composition.

Similar deductions to those made for pasture-fed herds may be made for supplemented herds. For each classification, the lowest producing herd receiving a supplement had, with only two exceptions, a protein level in the diet at least equal to the protein level in the selected pasture. On the basis of digestibility data of Morrison (1937) and Schneider (1947), it could be expected that the total digestible nutrients in supplements would be at least equal to the total digestible nutrients in selected pasture. The two exceptions were Herds 3 and 5 in Table 5, where protein was low in the supplement and where low protein intake could have limited productivity.

For the majority of herds, irrespective of whether they are of high or low productivity, there is a decline in autumn/spring from the summer level of production. For lower producing herds in each class of country, this must be ascribed largely to low feed intakes, although high energy costs of grazing may be contributing. For higher producing herds in each class of country, this seasonal decline must be due primarily to insufficient total energy from limited dry-matter intake, an insufficient total digestible nutrient content of pasture, and/or high energy cost of grazing.

On most properties in winter there was an abundance of low quality, mature grass, but sparse, short, green pasture was preferentially grazed. On some properties of low productivity the only feed available was short pasture. Thus field evidence supports the analytical evidence of low feed intakes as well as the high energy costs of grazing which must be expected under such conditions. A high energy supplement could correct all major factors responsible for seasonal decline in production in the area covered by the survey but may be uneconomical on the poorer classes of country.

(3) *Total Productivity.*—In each recorded herd in Tables 1 to 11, the production levels of cows lactating for 4 and 6 months are highly correlated with the production levels of cows lactating for 2 months ($r = 0{\cdot}96$ and $r = 0{\cdot}86$ respectively). Hence factors which determined the productivity of the freshest cows also determined the production of cows at all stages of lactation. Within herds, this relationship is similar throughout the year irrespective of the seasonal decline in production from autumn to spring.

As low productivity is essentially a nutritional problem, having been related to class of country, a low production in fresh cows must be due primarily to inadequate nutrition of heifers and/or cows in late gestation.

The minimum percentages of protein, phosphorus and calcium calculated in a manner similar to that used for calculating values in Tables 14, 15 and 16, and based on the recommendations of the National Research Council (1956) for heifers and cows in late gestation, are—

—	Protein	P	Ca
	(%)	(%)	(%)
Heifers (400 lb weight)	11·5	0·30	0·26
Cows in late gestation	9·5	0·15	0·16

The classes of country represented in Tables 4, 8, 10 and 12 are typical of areas used for heifers and dry cows in south-eastern Queensland. Even the poorest class, Table 9, in which selected pasture will not meet the maintenance requirements of adult cattle, is used alone or in association with other types of country. The summarized data in Table 13 emphasize that class of country, overall appearance of pasture and predominant pasture species grazed largely define the protein, phosphorus and calcium levels in pasture selected by cattle in south-eastern Queensland. In dry seasons, these levels must be expected to fall to at least the lowest levels recorded during this survey for each class of country. Thus on the classes of country represented in Tables 4, 8, 10 and 12, the protein, phosphorus and calcium levels in selectively grazed pasture would be inadequate for long periods of the year for cows in late gestation and would almost never meet the optimum requirements for heifers.

On many properties neither dry cows nor heifers are supplemented. The higher productivity achieved on better classes of country has been due partly to a better plane of nutrition in the milking herd and partly due to better feeding of dry cows, if only by bringing them into milking cow paddocks for some weeks prior to calving. However, it is most evident from this survey that a major cause of low total productivity of herds in south-eastern Queensland is inadequate nutrition of heifers and dry cows.

V. CONCLUSIONS

On the basis of this survey, three broad conclusions are apparent with regard to the correction of the low productivity of dairy cattle in south-eastern Queensland. These are:

(1) The need for optimum feeding of dry cows and heifers.

(2) The importance of the selection of a type of supplement which with pasture will provide enough protein, minerals and energy to maintain production at the summer level. The farm classifications may be used to assess the protein, phosphorus and calcium levels in selected pasture; published tables on the chemical compositions of feeds may be used to assess the feeding value of supplements.

(3) The significance of the essentially agricultural classifications as a guide not only to the nutritive value of the selected pasture but also to extending to other properties agricultural practices which have proved both effective and economical in lifting production within a classification type.

VI. ACKNOWLEDGEMENTS

The author is indebted to Mr. A. Hegarty (Agrostologist, Division of Plant Industry) for advice in classifying farm types, to officers of the Cattle Husbandry Branch who were associated with the collection of some of the data, and to Mr. S. E. Pegg (Chief Adviser, Herd Recording) who made herd records available.

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TEMPERATURE VARIATIONS IN FARM MILK TRANSPORTED UNDER SUBTROPICAL CONDITIONS

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SUMMARY

The mean temperature increase in milk during transport in cans in motor vehicles for 25-40 miles from farm to factory, with air temperatures of 73.2-86.0°F, was 6-10°F. A total of 7.3 per cent. of samples exhibited an increase of 20° or more, 29.2 per cent. an increase of 10-19°, 54.3 per cent. an increase of 0-9°, and 9.2 per cent. a temperature decrease during transit.

For milk in cans completely protected from air currents, rate-of-heating constants were 0.0096 and 0.0118, in comparison with 0.0223 for milk in cans transported in a vehicle with an open-slat superstructure. Temperature increases of milk in sealed vehicles were approximately 2-4°F lower than those of milk in open-slat vehicles.

Can position in the vehicle affected the temperature change. Milk temperature of "inside" cans increased at a lower rate than that of milk in "outside" cans.

I. INTRODUCTION

In countries where dairying is conducted under warm climatic conditions the keeping quality of milk is severely lowered unless the milk is cooled to a low temperature immediately after it is produced and then stored at low temperature.

Considerable information is available on the effects of health of cow, cleanliness of equipment, preparation of the cow, milking techniques, and temperature of cooling and storage on milk quality (Kelly and Clement 1931; Roadhouse and Henderson 1941; Harvey and Hill 1946; Cronshaw 1947). However, little work has been reported on the influence of transport conditions in hot climates on the temperature of milk. While producers have been advised of the advantages of cooling to and storage at low temperature on the farm, the importance of preventing a rise in temperature after the dairy produce has left the farm has not received the same attention.

Abraham and Outwater (1944) found under conditions of transport in New York State an average temperature increase in night's milk to New York from 47·8° to 52·2°F in uncooled vehicles and an average increase from 52·7° to 52·8°F in iced vehicles.

Pesk (1944), after examining the influence of temperature in open and closed truck hauling in the U.S.A., reported marked differences in internal

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temperatures between the two constructions. Floor temperatures of 70°F in the closed vehicle and 88°F in a partially open vehicle operating under similar atmospheric conditions were obtained. Temperatures in the partially open vehicle were found to vary with wind direction.

Working in England, Brindley, Scarlett, and Rowlands (1946) reported a change of 20°F in the temperature of milk in cans standing in the sun for six hours and a change of 10°F for cans stored in the shade during summer months. Jenkins, Reeve, and Provan (1949) investigated the effect of transport in open and enclosed vehicles, also in England. They stated that no useful purpose is served by protecting the cans from the direct heat of the sun during transit, irrespective of the temperature of the milk when cooled.

Brandsma and Hunt (1959) determined the temperature rise of refrigerated milk transported in closed, insulated trucks under atmospheric conditions ranging from 80° to 93°F. They concluded that the temperature of neighbouring cans, atmospheric temperature and length of time in transit are influencing factors.

In Queensland, Smythe (1945), investigating the influence of the "can factor" on milk quality using milk samples in tubes as controls, reported that time and duration of transport affected the fall in reduction of methylene blue times in a significant manner. Schubert (1956), correlating the quality of milk received in Brisbane milk factories with distance of transport and month of the year, reported that air temperature was an important factor in milk quality, and stressed the need for temperature control during storage on the farm and in transit.

In view of the increasing adoption of farm refrigeration for market milk supplies in Queensland, the investigation reported here was undertaken to determine the variations in milk temperature during transit under summer conditions from farm to factory in vehicles of variable construction.

No attempt was made to determine the keeping quality of experimental samples by the recognized milk quality tests because of the unknown history of the milk before pick-up and the difficulties associated with eliminating the influence of the "can factor" on quality.

II. METHODS

(a) Sampling Conditions

Temperature recordings were made on the same day at approximately fortnightly intervals during the period October 1957 to March 1958. The summer period was selected for sampling as the high atmospheric temperatures provided the most severe conditions for evaluating transport in covered vehicles.

The first can was loaded on to vehicles between 6.45 a.m. and 7.15 a.m. and vehicles arrived for unloading at processing depots between 10.00 a.m. and 10.45 a.m. Due to congestion at the factory it was usual for vehicles to stand for 1–1½ hr in the sun prior to unloading. In the case of Vehicles A and B, temperature recordings on closed vehicles were made on immediately succeeding days to the open-vehicle trial.

Recordings were made on a total of 1,671 cans.

(b) Vehicle Construction

McDowall (1953) and McWhirter (1956) have reported that accepted practice throughout New Zealand and Australia is for authorities controlling the production and supply of dairy produce to require vehicles transporting produce in cans to be covered to provide protection from direct sunlight on can surfaces.

The relevant regulation of *"The Dairy Produce Acts,* 1920 *to* 1959," of Queensland is as follows:

"Reg. 128. The owner of a conveyance used for the carriage of cream or milk to any other conveyance, butter factory, cream or milk depot, railway station, or railway siding, or other dairy produce premises, shall provide such firstmentioned conveyance with a permanent cover of wood, approved canvas, or other approved material which shall effectively protect from the sun's rays the dairy produce carried upon such firstmentioned conveyance. Such cover shall be not more than 5 feet 6 inches from the floor of the conveyance, with not less than 12 inches overhang on the sides and at the back. Where the cans are carried in two tiers there shall be a space of at least 3 inches clear above the bottom can and at least 9 inches clear between the top can and the roof. The sides shall be constructed of an approved louvre or boarded with timber not less than one-half inch thick, with a space of at least one-half inch between each board to a height of 3 feet 6 inches from the floor of the conveyance. The back shall be constructed in a like manner, and be in the form of a moveable tailboard: Provided that where milk or cream is conveyed by the person producing such milk or cream, this regulation shall not apply if a cover of canvas or other approved material is used to effectively protect such milk or cream against the sun's rays; such cover shall be affixed in such a manner as to afford ventilation between the can and such cover."

As such legislation only prescribes minimum requirements, a variety of interpretations of vehicle construction is possible. An earlier independent survey indicated that this variation in construction occurred in Queensland.

The regulations under Acts of various Australian States governing dairy produce do not prescribe conditions for the control of the movement of air around the cans during transit. Under weather conditions prevailing in Queensland during

summer, where mid-morning temperatures approximate 85–95°F on many days, such air movement around cans containing refrigerated milk causes marked increases in temperature. Vehicle construction in this investigation was modified therefore to determine the influence of various forms of covering on the extent of milk temperature increase during transit.

Five vehicles providing seven different types of conditions were used initially, but owing to difficulties in obtaining similar pick-up temperatures with one pairing (Vehicles D and E), this section of the trial was discontinued after five comparisons. The modified pick-up system was restricted to three vehicles providing five different conditions of transit.

TABLE 1

Details of Transport Conditions

Vehicle	Maximum Load (cans)	Classification	Transport Distance (miles)	Transport Time	Construction
				hr min	
A	Lower = 90 Upper = 36	Open ..	37	3 30	Conventional slat construction Hood 6 ft from tray. Open rear
A1	„	Closed ..	„	„	Vehicle A completely enclosed with canvas hood
B	Lower = 90 Upper = 72	Closed (partially)	40	2 50	Complete seal of sides. Front end sealed to lid of can in upper tier. Hood 5 ft 9 in. from tray. Open rear
B1	„	Closed ..	„	„	Vehicle B completely enclosed with light timber and rear flap
C	Lower = 90 Upper = 66	Closed (partially)	38	4 00	Complete seal of sides and ends to shoulder of can in upper tier. Hood 5 ft 9 in. from tray
D	Lower = 78 Upper = 42	Open ..	31	3 00	Conventional slat construction similar to A. Hood 5 ft 9 in. from tray. Open rear
E	Lower = 78 Upper = 30	Closed ..	26	3 00	Completely enclosed construction with timber similar to B1. Hood 5 ft 9 in. from tray. Open rear

Details of vehicle construction, together with loadings, distances and transit times, are summarized in Table 1. Vehicles are illustrated in Figures 1–7.

On arrival at the factory, a visual examination for excessive dust contamination was made of cans in closed vehicles.

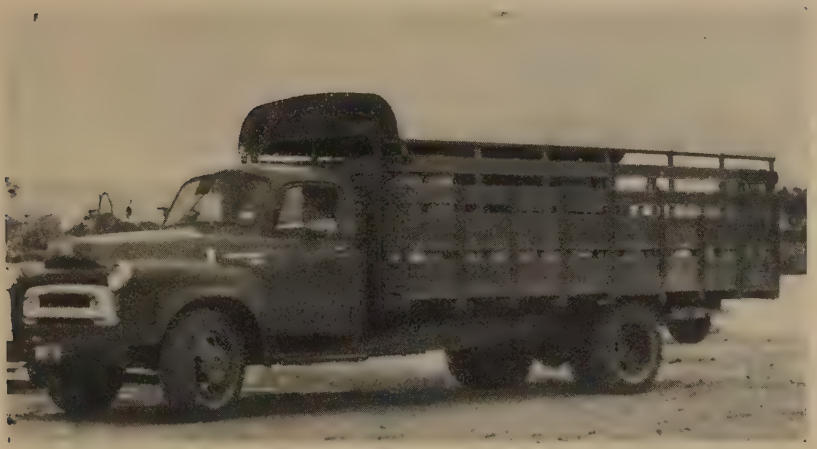

Fig. 1.—Vehicle A. Conventional slat cover typical of milk transport vehicles. Note that this structure permits air movement around cans.

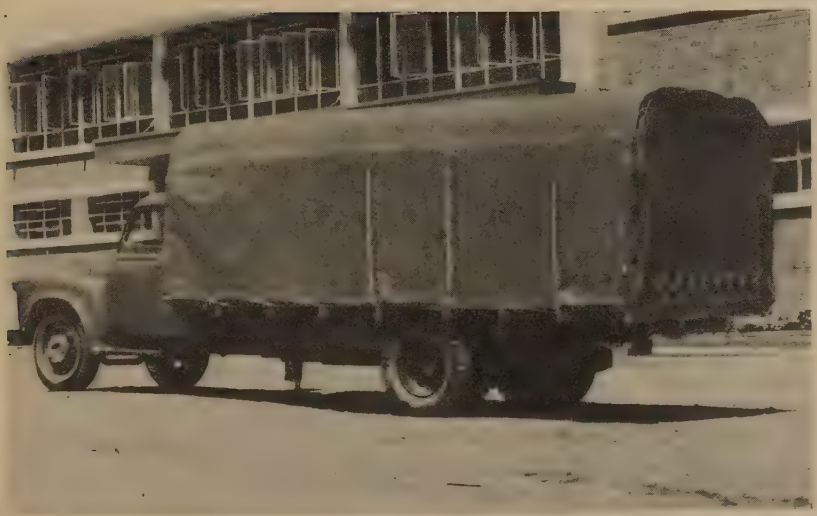

Fig. 2.—Vehicle A enclosed with a heavy-duty canvas canopy (Vehicle A1). Both front and rear ends are protected with separate flaps.

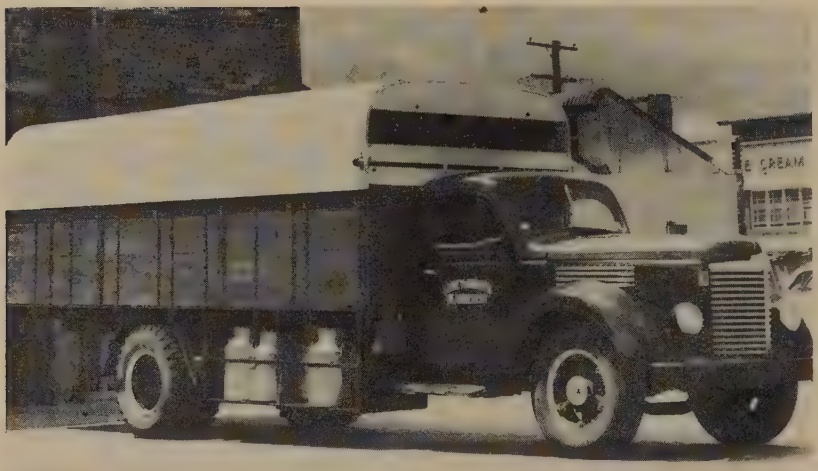

Fig. 3.—Vehicle B. Timber sides of vehicle sealed to provide complete protection from draught. Open slat in front end permits air movement above surface of cans.

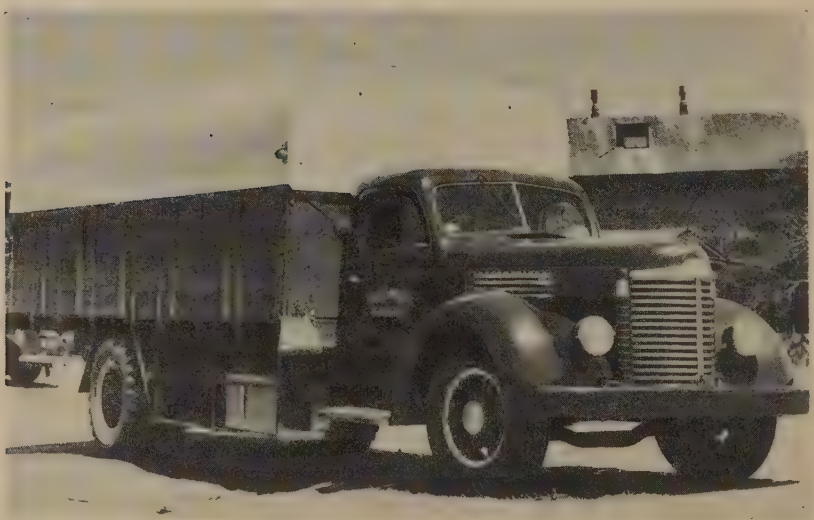

Fig. 4.—Vehicle B completely sealed with a timber insert at front end and a canvas flap on rear end (Vehicle B1)

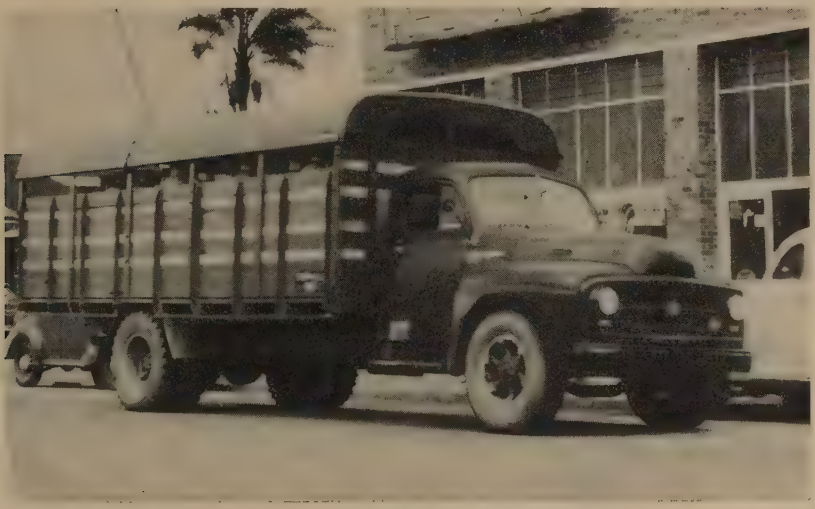

Fig. 5.—Vehicle C. Timber inserts fitted to conventional structure to restrict air movement to upper tier of cans.

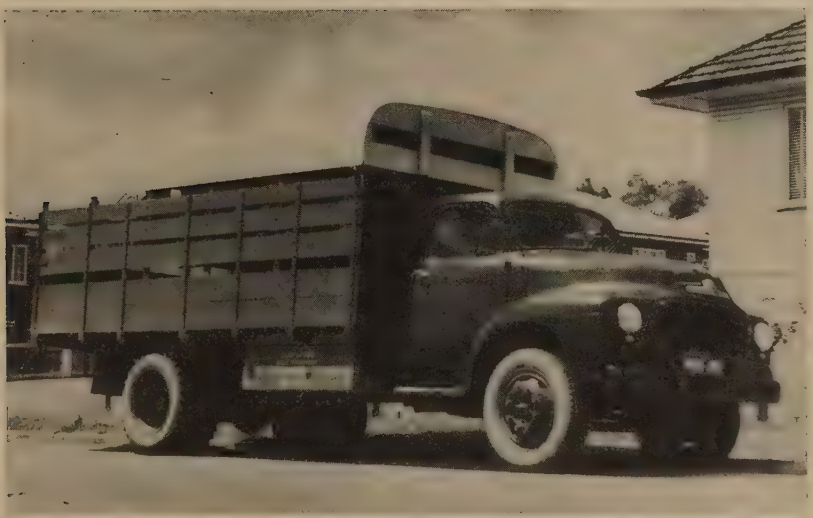

Fig. 6.—Vehicle D. Small-capacity vehicle of conventional structure. Compare with Vehicle A (Fig. 1).

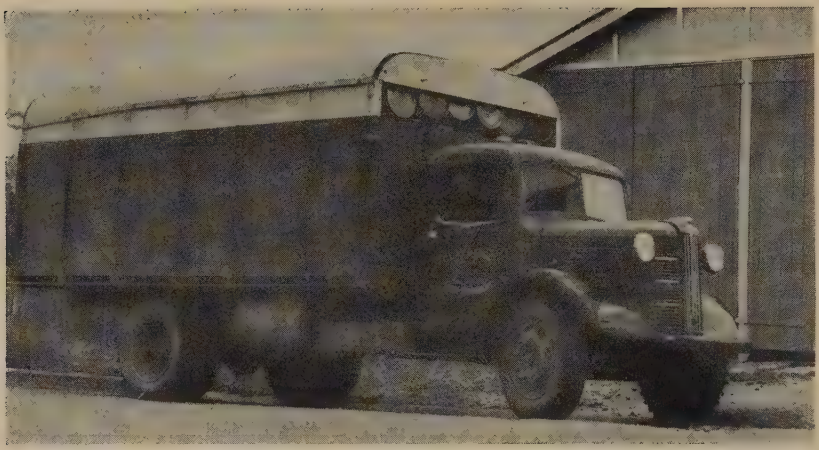

Fig. 7.—Vehicle E. Small-capacity vehicle with completely sealed sides. During transit the front end was sealed with a timber insert but rear end remained open.

(c) Milk Temperature

The temperature of the milk was recorded at the point of pick-up at the farm gate and at the time of tipping at the processing factory. The temperature rise in milk which occurred as a consequence of vehicles standing in the sun awaiting tipping was included therefore in the overall temperature rise. This was unavoidable, as it was not possible to record all sample cans until the load was discharged for tipping. However, a random sample of milk temperatures was recorded on a small number of cans on arrival at the factory for comparison with tipping temperatures.

Milks were selected to embrace the temperature range of 40–80°F to ensure that refrigerated evening supplies and uncooled morning samples were examined. Difficulty was experienced in securing a large number of samples in the above 80°F group.

Temperatures were recorded to the nearest °F.

(d) Can Position

Sample cans were selected according to a predetermined position in the vehicles. Cans 1, 3, 4 and 6 of Rows 1, 5 10 and 15 were recorded in the lower tier, and Cans 1, 3, 4 and 6 of Rows 1, 5 and 10 in the upper tier. In addition, Can 1 in adjacent rows was recorded. In the majority of instances "cooled" and "uncooled" milks were placed together. The positioning of cans is illustrated in Figure 8. Similar positioning of sample cans was arranged for trucks of covered construction in comparative trials.

Positioning of sample cans enabled groupings of "inside" and "outside" cans to be arranged when analysing results for temperature differences.

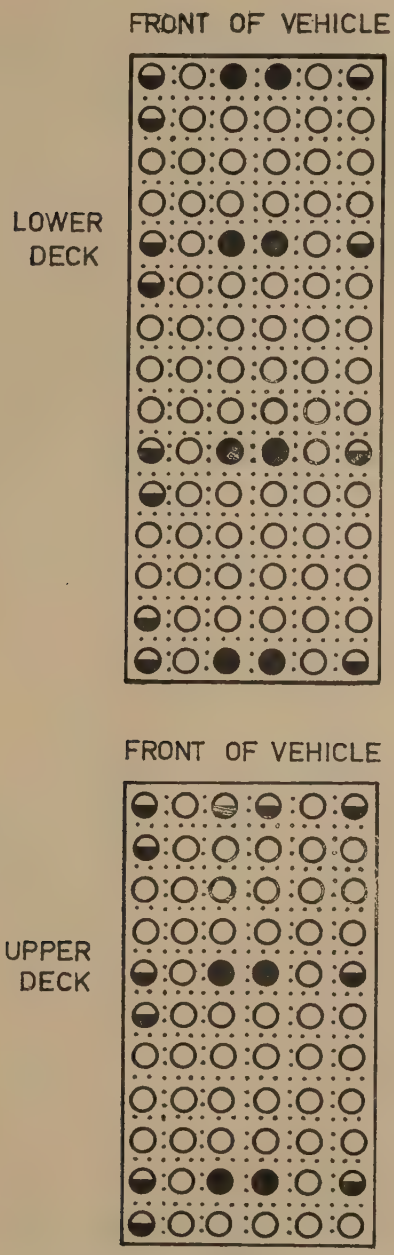

Fig. 8.—Diagram showing position of sample cans. The filled circles were regarded as "inside" positions.

TABLE 2

Mean Milk Temperatures, Vehicles A and A1

Temperature Range (°F)	Vehicle A (Open)					Vehicle A1 (Closed)					Significance of Difference between Means
	Cans		Mean Temperature of Milk (°F)			Cans		Mean Temperature of Milk (°F)			
	No.	%	At Farm Gate	On Factory Platform	Increase	No.	%	At Farm Gate	On Factory Platform	Increase	
50 and below ..	58	23·5	46·9	63·8	16·9 ± 0·68	57	23·1	46·2	58·8	12·6 ± 0·60	Sig. (1%)
51–60	90	36·4	55·3	67·4	12·1 ± 0·47	85	34·4	55·9	63·2	7·3 ± 0·36	Sig. (1%)
61–70	87	35·2	64·9	70·4	5·5 ± 0·31	97	39·3	64·4	68·0	3·6 ± 0·61	Sig. (1%)
71–80	10	4·1	72·6	74·6	2·0 ± 0·54	8	3·2	72·3	73·8	1·5 ± 0·52	Not sig.
81 and over ..	2	0·8	81·5	82·5	1·0	..	0	..	..	..	..
Total ..	247		57·6	68·0	10·4 ± 0·40	247		57·5	64·4	6·9 ± 0·30	Sig. (1%)

TABLE 3

Mean Milk Temperatures, Vehicles B and B1

Temperature Range (°F)	Vehicle B (Partially Closed)					Vehicle B1 (Closed)					Significance of Difference between Means
	Cans		Mean Temperature of Milk (°F)			Cans		Mean Temperature of Milk (°F)			
	No.	%	At Farm Gate	On Factory Platform	Increase	No.	%	At Farm Gate	On Factory Platform	Increase	
50 and below ..	133	39·7	44·7	61·4	16·7 ± 0·55	129	38·5	44·5	57·7	13·2 ± 0·37	Sig. (1%)
51–60	61	18·2	56·1	64·2	8·1 ± 0·44	69	20·6	55·6	62·6	7·0 ± 0·38	Not sig.
61–70	86	25·7	65·8	69·2	3·4 ± 0·29	77	23·0	65·5	67·5	2·0 ± 0·22	Sig. (1%)
71–80	50	14·9	75·8	74·9	−0·9 ± 0·28	51	15·2	75·7	74·4	−1·3 ± 0·21	Not sig.
81 and over ..	5	1·5	81·8	81·0	−0·8 ± 0·90	9	2·7	81·4	78·0	−3·4 ± 0·53	Sig. (5%)
Total ..	335		57·4	66·2	8·8 ± 0·45	335		57·4	64·1	6·7 ± 0·36	Sig. (1%)

(e) Weather Conditions

As movement of air through the vehicles was the main factor causing milk temperature increases, air temperatures were recorded at hourly intervals while the vehicles were in motion. These records were checked against official meteorological recordings in the producing area. The air temperature at 9.00 a.m. was regarded as typical of weather conditions during transit unless wide hourly fluctuations occurred. This time approximated the middle of the collection journey.

III. RESULTS

(a) Increase in Milk Temperature

Results of mean temperature recordings on milk when loaded into the transport vehicle at the farm gate, and on unloading on the factory receival platform, are listed for the several vehicles in Tables 2–6. Initial temperatures were grouped in 10°F ranges from "50°F and below" to "81°F and above." Differences in milk temperature increases between farm and factory for open and closed vehicles were examined for significance.

TABLE 4

Mean Milk Temperatures, Vehicle C

Temperature Range (°F)	Vehicle C (Partially Closed)				
	Cans		Mean Temperature of Milk (°F)		
	No.	%	At Farm Gate	On Factory Platform	Increase
50 and below ..	58	19·2	45·4	64·8	19·4 ± 0·69
51–60	90	29·7	55·9	64·5	8·6 ± 0·53
61–70	101	33·3	63·8	67·7	3·9 ± 0·30
71–80	41	13·5	74·0	74·0	0 ± 0·36
81 and over ..	13	4·3	82·5	80·3	−2·2 ± 0·49
Totals ..	303		60·1	67·6	7·5 ± 0·45

(i) Vehicle A

This trial provided a comparison between the conventional open-slat construction (see Reg. 128) and the completely closed structure. Closure was obtained by fitting a canvas hood over the existing superstructure and fitting a rear canvas flap.

TABLE 5

Mean Milk Temperatures, Vehicle D

Temperature Range (°F)	Vehicle D (Open)				
	Cans		Mean Temperature of Milk (°F)		
	No.	%	At Farm Gate	On Factory Platform	Increase
50 and below ..	39	38·2	45·5	62·5	17·0 ± 0·81
51–60	31	30·4	54·7	62·3	7·6 ± 0·45
61–70	8	7·8	63·8	66·8	3·0 ± 1·01
71–80	11	10·8	77·7	76·0	−1·7 ± 0·59
81 and over ..	13	12·8	83·1	78·6	−4·5 ± 0·92
Total ..	102		58·0	66·3	8·3 ± 0·87

TABLE 6

Mean Milk Temperatures, Vehicle E

Temperature Range (°F)	Vehicle E (Closed)				
	Cans		Mean Temperature of Milk (°F)		
	No.	%	At Farm Gate	On Factory Platform	Increase
50 and below ..	41	40·2	44·9	56·9	12·0 ± 0·73
51–60	35	34·3	56·3	62·9	6·6 ± 0·70
61–70	11	10·8	63·7	66·2	2·5 ± 0·56
71–80	15	14·7	74·3	73·9	−0·4 ± 0·64
81 and over ..	..	..	..	..	..
Total ..	102		55·2	62·5	7·3 ± 0·59

Mean milk temperature increased from 57·6° to 68·0°, an increase of 10·4°, in the open vehicle, and from 57·5° to 64·4°, an increase of 6·9°, with the enclosed superstructure (Table 2). This difference in "all cans" mean increase was significant statistically, as were the group differences in the temperature ranges "50 and below," "51–60" and "61–70."

The percentage distribution of individual can increases, grouped in 5° ranges, is listed in Table 7. These results indicate a wider range of distribution in milk from the open vehicle than with the closed construction. With the closed vehicle 25·5 per cent. of milks exhibited an increase in the "10° and over" range, compared with 49·0 per cent. of milks in the conventional open-slat construction.

(ii) Vehicle B

This trial provided a comparison between a partially enclosed and a completely enclosed vehicle. Complete closure was obtained by inserting a timber facing on the open front end and fitting a rear canvas flap.

Mean milk temperature increased from 57·4° to 66·2°, an increase of 8·8°, in the partially enclosed vehicle, and from 57·4° to 64·1°, an increase of 6·7°, in the completely closed type (Table 3). The difference in the "all cans" mean temperature increase between trucks was significant, as were the group differences in the temperature ranges "50 and below," "61–70" and "81 and over."

A comparison of the mean temperature increase of all milk in Vehicle A (closed) and Vehicle B (closed) reveals a close relationship of 6·9° and 6·7° respectively, which increases were not significantly different. There was a significant difference in the mean temperature increases obtained in the partially closed Vehicle B (8·8°) and the conventional open Vehicle A (10·4°).

In agreement with the "all can" difference in mean temperature increase between partially closed and closed vehicles, the percentage of individual can increases in the low temperature grouping was higher with closed construction than in the partially closed type (Table 7). This difference in distribution was not as marked as that which occurred between open and closed superstructure with Vehicle A.

A feature of this trial was the appreciable percentage of cans which exhibited a temperature decrease during transit, viz. 11·0 in the partially closed vehicle and 14·9 with the completely sealed construction. Table 3 shows that 15–20 per cent. of sample cans had initial milk temperatures approximating or exceeding atmospheric conditions (see Table 11), which circumstances could account for this slight cooling during transit. The influence of cooler neighbouring cans on individual temperature decreases is advanced as a further factor, especially in those instances where initial milk temperatures were below air temperature.

(iii) Vehicle C

In this trial mean milk temperature increased from 60·1° at the farm gate to 67·6° on tipping at the factory, a mean increase of 7·5° during transit (Table 4). This vehicle was partially enclosed, protection afforded being intermediate between Vehicle A (open) and Vehicle B (partially closed).

TABLE 7

Percentage Distribution of Increases in Milk Temperature of all Vehicles During Transit

Range of Temperature Increase (°F)	Vehicle A		Vehicle B		Vehicle C	Vehicle D	Vehicle E	Can Position		All Vehicles
	Open	Closed	Partially Closed	Closed	Partially Closed	Open	Closed	Inside	Outside	
Decrease ..	0·4	0·4	11·0	14·9	10·6	23·5	8·8	11·3	8·0	9·2
0– 4 ..	19·0	38·9	23·6	28·1	36·6	9·8	28·5	38·6	22·2	27·9
5– 9 ..	31·6	35·2	23·9	21·8	22·1	25·5	29·4	26·6	26·2	26·4
10–14 ..	21·1	17·8	17·9	22·1	9·6	16·7	20·6	12·9	20·5	17·8
15–19 ..	19·8	6·1	11·3	9·5	11·9	10·8	8·8	8·4	13·0	11·4
20–24 ..	4·9	1·2	7·2	3·6	5·9	12·7	3·9	1·9	6·9	5·1
25 and over ..	3·2	0·4	5·1	..	3·3	1·0	..	0·3	3·2	2·2

A smaller percentage of cans in the "50 and below" group resulted in the "all cans" mean farm milk temperature being higher than with Vehicle B.

A slightly increased percentage of cans exhibited low temperature increases with this trial in comparison with Trial B, due to the lower proportion of cans in the "50 and below" and "51–60" farm temperature ranges. As with Trial B, several cans exhibited initial milk temperatures in excess of 70° and subsequent cooling occurred during transit; 10·6 per cent. of all cans decreased in temperature between the farm gate and the factory.

(iv) Vehicle D

Mean milk temperature increased from 58·0° to 66·3°, an increase of 8·3° between farm pick-up and factory tip (Table 5).

The percentage of cans in the lower ranges of increases in temperature during transit ranged from 33·3 for "4 and below" to 13·7 for "20 and above." A comparison of this percentage distribution with the other open vehicle in the investigation, Vehicle A (open), indicated a wide difference in the lower ranges, Vehicle D exhibiting an increased percentage. This could be accounted for by the larger number of cans in the higher initial temperature grouping. Of all vehicles, this vehicle transported the greatest portion of milk "greater than 70°" and it exhibited the greatest percentage of milk which showed temperature decrease in transit.

(v) Vehicle E

In this trial mean milk temperatures increased from 55·2° at the farm to 62·5° at the factory, an increase of 7·3° (Table 6). Complete closure was obtained by inserting a timber facing on the open front end, the canopy sides already being sealed. No rear protection was fitted on this vehicle.

Because initial milk temperature was lower in this vehicle in comparison with milk on Vehicle D, higher increases would be expected during transit if the vehicle had been uncovered. However, it was found that the percentage distribution of individual cans increases in the lower ranges for milk in Vehicle E was much greater than with milk in Vehicle D. Of the cans in Vehicle E, 87·3 per cent. recorded increases of "14° and below" compared with 75·4 per cent. of cans in this range for Vehicle D, suggesting a beneficial effect from closing.

(b) Rate of Temperature Increase

As the rate of heating of a body under given conditions is proportional to the temperature difference between the body and its surroundings, individual can temperatures were related to atmospheric temperatures, distance of transport and

types of covering. All milk temperatures for each vehicle at the farm and the factory were analysed according to the following formula:—

$$k = \frac{1}{m} \log_e \frac{\theta_A - \theta_O}{\theta_A - \theta_1}$$

where k = heating constant/mile °F temperature difference between milk and atmosphere

m = miles in transit

θ_A = mean air temperature

θ_O = temperature of milk at farm

θ_1 = temperature of milk at factory

In this grouping no allowance was made for the influence of can position on temperature, as this effect should be similar for comparative recordings. It was not possible in the analysis to exclude the influence of neighbouring cans on individual temperature changes. It was noted that 161 cans of a total of 1671 examined recorded either a temperature decrease during transit, initial temperatures in excess of air temperatures or final temperatures in excess of air temperatures. These 161 cans were excluded in calculating the "k" value for vehicles.

Heating factors within the range ·0096 to ·0223 were obtained from the seven different constructions examined. Results are listed in Table 8. A marked difference in the rate of heating was evident between the vehicles of the conventional slat superstructure and the vehicles completely enclosed. In the former instance, with Vehicle A, a value of ·0223 was obtained, in contrast to factors of ·0096 and ·0118 when milk was transported in closed Vehicles B and A respectively.

The results obtained with Vehicle C (partially closed) could be considered relatively high although the protection afforded to cans was not as complete as with Vehicle B (partially closed).

(c) Position of Can

While initial farm and atmospheric temperatures are important in determining the final milk temperature after transport, the position of a can on the transport vehicle must be considered.

To examine the effect of "inside" and "outside" can positions on changes in milk temperature, results were segregated into "inner" and "outer" groupings and analysed. Rate-of-heating constants were also determined for cans in these positions to ascertain any variations in heating rate because of differing conditions of air movement around the external surface of the can. Differences in the rate

of heating milk in "outside" and "inside" cans were found, the variation being widest in open vehicles. The results for the individual trucks are tabulated in Tables 8 and 9.

TABLE 8

Rate-of-Heating Constant for Cans in Various Positions

Vehicle	Construction	Total Cans Recorded	"k" Factor					
			Inside Cans		Outside Cans		All Cans Analysed	
			No.	Factor	No.	Factor	No.	Factor
B1	Closed	335	98	·0078	188	·0104	286	·0096 ± ·0003
A1	Closed	247	82	·0096	164	·0128	246	·0118 ± ·0002
B	Partially Closed ..	335	111	·0104	189	·0155	300	·0136 ± ·0005
E	Closed	102	29	·0112	64	·0155	93	·0142 ± ·0010
D	Open	102	23	·0109	55	·0168	78	·0150 ± ·0009
C	Partially Closed ..	303	96	·0144	166	·0232	262	·0203 ± ·0008
A	Open	247	82	·0151	163	·0258	245	·0223 ± ·0008
		1,671	521		989		1,510	

The benefit of the additional protection afforded with closed superstructure to both inside and outside cans in trials on Vehicles A1 and B1 was reflected in the lower actual temperature increases and the lower final temperatures recorded for cans examined in these vehicles in comparison with results from Vehicles A and B.

(d) Temperature Increase on Standing

To determine the temperature increases in milk while vehicles were awaiting receival, the temperatures of a small number of cans were taken immediately on arrival and compared with temperature of milk at tipping. Results are listed in Table 10. The limited number of samples did not permit any differentiation between open and closed superstructure but only indicated a mean rise of the order of 1–2° for the 1–1½ hr standing period. This low rise supports the premise that where standing time is not excessive the major cause of temperature variation during transit is the movement of air through the vehicle.

(e) Atmospheric Temperature

Mean air temperatures at 9.00 a.m. on collection days are listed in Table 11. Air temperatures for the 6 months' sampling period averaged 80·7°, with a range of 73·2° to 86·0°, the peak occurring in the month of November. Air temperatures when comparing covered and uncovered vehicles on succeeding sampling days were not identical, but the mean temperature for the sampling period was slightly higher on the days when covered vehicles were examined; this provided somewhat severer testing conditions.

TABLE 9

Mean Milk Temperature According to Position of Cans (All Vehicles)

Vehicle	Construction	Inside Position				Outside Position			
		No. of Cans	At Farm Gate (°F)	On Factory Platform (°F)	Increase (°F)	No. of Cans	At Farm Gate (°F)	On Factory Platform (°F)	Increase (°F)
A	Open	83	58·3	66·5	8·2 ± 0·65	164	57·2	68·8	11·6 ± 0·48
	Closed	82	58·0	63·5	5·5 ± 0·47	165	57·3	64·8	7·5 ± 0·38
B	Partially Closed ..	126	58·7	65·2	6·5 ± 0·58	209	56·6	66·9	10·3 ± 0·61
	Closed	126	59·2	63·9	4·7 ± 0·53	209	56·3	64·2	7·9 ± 0·48
C	Partially Closed ..	113	62·1	67·0	4·9 ± 0·52	190	58·9	67·9	9·0 ± 0·61
D	Open	31	59·2	65·2	6·0 ± 1·20	71	57·4	66·7	9·3 ± 1·11
E	Closed	31	53·9	60·6	6·7 ± 0·92	71	55·7	63·2	7·5 ± 0·76

TABLE 10

Milk Temperature Increase (°F) in Open and Closed Vehicles Standing for 1–1½ Hours

Position of Cans	Construction	No. of Cans	Temperature on Arrival (°F)	Temperature at Tip (°F)	Temperature Increase (°F)
Inside ..	Closed ..	12	61·2	61·8	0·6
	Open ..	9	59·2	60·8	1·6
Outside ..	Closed ..	24	63·4	64·2	0·8
	Open ..	18	67·4	68·8	1·4
All Cans		63	63·5	64·6	1·1

TABLE 11

Air Temperature (°F) at 9.00 a.m. on Collection Days for all Vehicles

Month	Mean Air Temperature (°F)							Mean Monthly Air Temperature (°F)
	Vehicle A		Vehicle B		Vehicle C	Vehicle D	Vehicle E	
	Open	Closed	Partially Closed	Closed	Partially Closed	Open	Closed	
October	72	77	75	77	71	..	..	74·4
	78	75	78	76	83	78	80	78·3
November	82	86	81	82	73	82	82	81·1
	92	86	87	85	82	88	82	86·0
December	86	82	82	84	74	88	89	83·6
	76	86	84	86	80	89	87	84·0
January	84	81	82	87	81	..	..	83·0
February	84	86	83	84	82	..	..	83·8
	78	75	79	75	73	..	..	76·0
March	78	80	80	82	80	..	..	80·0
	70	74	74	78	70	..	..	73·2
Mean Trial Air Temperature (°F) ..	80·0	80·7	80·5	81·5	77·1	85·0	84·0	80·7

(f) Dust Contamination

An examination of cans for dust contamination when transported in sealed vehicles indicated that the entry of dust from the rear of the vehicle could reach serious proportions unless the protective covering was close fitting. On those collection days when a rear flap was not used, heavy dust deposits entered and settled on the last 2–4 rows of cans. This was particularly evident for trips with closed Vehicles A1 and B1 but not so severe with Vehicle E. Dust entered as the vehicles slowed to a standstill for can collection. However, when the rear flap was in position contamination was only slight. A loss in freedom of movement for the driver during loading was apparent where completely enclosed vehicles were operating. The accepted practice of loading vehicles from the rear could require modification where complete enclosure was adopted.

Vehicle B, which during normal collection was partially enclosed and therefore permitted a stream of air to pass above the cans in the top tier, was not contaminated to any marked degree.

IV. DISCUSSION

While initial bacterial contamination and subsequent development of such organisms under the conditions of temperature and storage of the farm exert the major influence on milk quality, the continuation of undesirable time/temperature storage conditions during transit can be important. The results obtained in this investigation showed:—

(1) Milk cooling methods on the farm as indicated by pick-up temperatures were variable.

(2) Temperatures which prevailed in milk during transit coincided with the critical level for quality deterioration.

(3) Temperature increase was less when the superstructure of the transport vehicle was modified to minimize air movement around the cans during transit.

(a) Farm Temperature

Of the 1671 cans examined, the percentages in the temperature groupings were—

Temperature Range (°F)	Percentage of Sample
50 and below ..	30·8
51 to 60	27·6
61 to 70	28·0
71 to 80	11·1
81 and over ..	2·5

Difficulty was experienced in obtaining a proportional number of cans in the "81 and over" group.

As milk temperatures below 70°F are only possible on most days under Queensland summer conditions by mechanical refrigeration, these results indicated that 86·4 per cent. of all samples had been subjected to such cooling. There was, however, an even distribution of cans in the temperature groupings from "below 50 to 70°", suggesting a wide variation in the effective utilization of cooling.

The incidence of 13·6 per cent. of cans with temperatures in excess of 70° suggested there was a group of producers whose production methods required revision if quality deterioration prior to arrival at the factory was to be minimized.

(b) Optimum Temperature Level

Smythe (1945), Morton and Vincent (1949) and Vincent (1952) reported that deterioration in milk quality as measured by the methylene blue test commences at 50°F and proceeds at an increasing rate as temperatures rise to 85–90°F. A second factor, the time of storage at the particular temperature level, was also related to quality deterioration. For 4 hours' storage, Vincent (1952) listed the following fall in reduction time of the methylene blue test based on (1) Smythe's investigations and (2) prediction from regression equations of Morton and Vincent.

Storage Temperature		Fall in Reduction Time (½ hr units) 4 hours' storage	
°C	°F	Smythe	Morton and Vincent
10	50	−0·3	1·2
15	59	1·0	3·3
20	68	4·4	5·7
25	77	7·2	8·1
30	86	10·6	10·6

Smythe also reported that as the storage temperature increased the rate of deterioration (fall in reduction time) was greater in good quality milks than in milks of inferior quality. Furthermore, this rate of deterioration with temperature increased as storage time was prolonged and such an added deterioration was greater when milk quality was high. However, no correlation was obtained between the initial quality of a milk and the effect of storage time.

Bakalor (1946) stated that at milk temperatures above 60°F quality deterioration is of serious proportions.

The mean milk temperature at the farm gate obtained in this investigation ranged from 55° to 60°, at which temperature rapid quality deterioration is initiated. A total of 69·2 per cent. of cans exhibited initial temperatures in excess

of 50°. The onset of spoilage would be of greater significance in those milks loaded first onto vehicles, such milk being of a longer post-cooling age before processing than milk collected towards the end of the route. In addition, this milk also approaches more closely to atmospheric temperatures during transit, as evidenced by calculations based on the rate of heating ("k" factor). For a milk with an initial temperature of 50°, transported 40 miles to the processing factory in a vehicle of conventional slat construction under a mean atmospheric temperature of 80°, using a heating constant value (k) of 0·021 it is estimated that the temperature on arrival at the factory platform would approximate 67·1°. In a vehicle completely covered the final temperature calculated using a constant of ·011 would approximate 60·7°. This factor is a guide to general conditions rather than a specific can constant because of the indeterminate influence of can neighbours. This influence of surrounding cans on temperature changes during transit raises the issue of whether a farmer supplying milk of high temperature should be permitted to benefit in terms of quality because his milk is adjacent to refrigerated supplies. The temperature decreases obtained in 9·2 per cent. of all cans examined indicates that such benefits do arise.

As with distance, can positioning was also found to influence the final temperature level of milk. Temperature increases in individual cans exhibited a wide fluctuation from "greater than 25°" to "less than zero" (temperature decrease). An examination of Table 7 reveals that a greater percentage of milk (23·1) in outside cans exhibited temperature increases in excess of 14° than did milk from cans in the inside position (10·6). This suggests that it is with individual cans that the influence of can position on temperature assumes importance. If transport conditions can be so arranged to afford added protection to such individual supplies, then modifications are warranted.

(c) Influence of Covering on Milk Temperature

It is considered that one of the prime purposes of covering vehicles should be to prevent the passage of hot air through the vehicle while in transit, thus minimizing the increase in temperature of those milks which had been previously cooled on the farm.

Jenkins *et al.* (1949) reported an average temperature increase of 2°F during transit in open vehicles and an increase of only 1°F when the vehicles were protected with tarpaulins. Results obtained in this Queensland investigation indicated "all can" mean increases ranged from 6·7 to 10·4°F in vehicles differing in construction. The greater percentage of cans in the lower initial temperature ranges (58·4 per cent. below 61°F) in this investigation, compared with 36 per cent. of cans in this range in the English trials, could account for this larger temperature increase.

McWhirter (1956) reported that "experiments revealed there is no rise in temperature of cream in summer weather when the cans are not covered provided that the vehicle is not stationary for longer than the time necessary for pick-up, i.e. 1–2 mins." No temperature results of these experiments were reported.

In Vehicle A it was possible to reduce the extent of temperature increase during transit between the open conventional slat superstructure and the completely sealed construction from 10·4 to 6·9°, a reduction of 3·5°. With modifications to Vehicle B, when a partially enclosed vehicle was completely sealed to prevent air movement around the cans, increases were reduced from a mean of 8·8° to 6·7°, a decrease of 2·1°.

Close agreement was obtained between temperature increase and final milk temperature for Vehicles A1 and B1 with completely sealed construction, indicating that enclosure with a removable canvas canopy provided protection similar to that obtained when the superstructure was composed of timber.

As indicated in Table 1, less protection was afforded to cans in Vehicle C, where only partial closing was provided, than to cans in Vehicle B (partially closed). Final mean milk temperature of 67·6° in the former vehicle in comparison with 66.2° for Vehicle B, when initial mean milk temperature was 2·7° higher and atmospheric temperature lower, suggests that the protection afforded when sides are timbered to can shoulder height is not as effective as when sides are sealed to protect the complete can. In this latter construction (Vehicle B), air movement was directed largely along the lower side of the top canopy and above the surface of the can.

Initial mean milk temperatures for Vehicle D (open) and Vehicle E (closed) were 58·0° and 55·2° respectively. This initial variation made it difficult to obtain a satisfactory comparison of the effectiveness of covering. Final mean temperatures, however, suggest that some benefit was obtained with milk in Vehicle E from the additional protection provided.

(d) Influence of Covering on Dust Contamination

McWhirter (1956) indicated "the incidence of dust from unsealed roads settling on cans is greater when a canopy type cover is used and is at a minimum when no cover at all is employed."

Results obtained in this investigation indicated that when movement of air through the vehicle was restricted the entry of dust from unsealed roadways increased. It was possible, however, with the provision of a canvas rear flap to prevent serious dust contamination of can surfaces. These results show that where enclosure of superstructure is recommended provision must be made also for sealing of the rear of the vehicle. Alternatively, if some air movement is considered desirable to provide a positive internal air pressure, construction should be so modified as to direct the air flow immediately below the protective canopy and not around the can surface.

(e) Aesthetic Considerations in Covering

The viewpoint of providing the maximum protection possible to a perishable product deserves consideration when high standards of production and storage are demanded of producers. It can be argued that for transport under subtropical weather conditions complete protection should be provided.

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INDUCTION OF FERTILITY BY COLCHICINE TREATMENT IN RONPHA GRASS, A STERILE INTERSPECIFIC PHALARIS HYBRID

Ronpha grass, a sterile natural hybrid of *Phalaris arundinacea* and *Phalaris tuberosa* var. *stenoptera,* was introduced to Queensland from South Africa, where it is used mainly as supplementary green grazing under irrigation for cattle and sheep during the winter months in the summer rainfall region. It has shown desirable characteristics under testing in the subtropical environment of Central Queensland.

Ronpha grass produces inflorescences in abundance over an unusually long period. This lengthening of the flowering period has often been observed in male-sterile plants belonging to pure species and interspecific and intergeneric hybrids. Flowering in Ronpha grass is very incomplete. Some florets exsert both anthers and stigmas quite normally, but the anthers are slender and often shrivelled, and they do not dehisce. Some florets do not exsert the stigmas nor the anthers and

Fig. 1.—Typical plants of Ronpha grass.

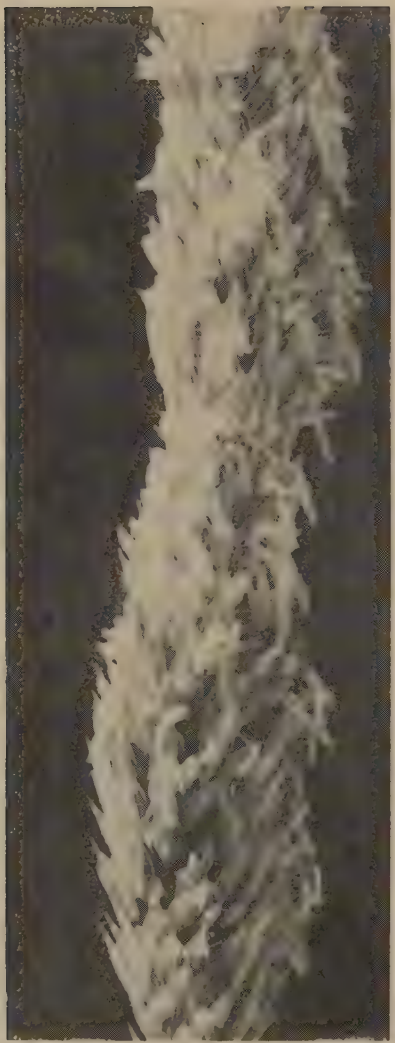

Fig. 2.—Normal anthesis of Ronpha grass after treatment with colchicine.

they shrivel *in situ* without liberating any pollen. This is in agreement with the incomplete flowering of the *P. arundinacea* x *P. tuberosa* hybrid produced by artificial hybridization by Jenkin and Sethi (1932).

Microscopic examination of the pollen grains of Ronpha grass at Biloela Regional Experiment Station has shown a small percentage (2–4), of stainable pollen. This hybrid, however, is functionally male-sterile owing to the non-dehiscence of the anthers. Over 3,000 seedheads were threshed by hand and no viable seed was obtained.

Hutton (1953) obtained the allopolyploids of *P. tuberosa* x *P. minor* and *P. coerulescens* x *P. minor* hybrids by cutting back growth to 2 cm from the ground and painting plants with an aqueous solution containing 0·2 per cent. colchicine and 0·1 per cent. spreader. This treatment was repeated on six successive days.

In the present study, clonal material of Ronpha grass was treated with a 0·2 per cent. aqueous solution of colchicine. The methods of application were as follows:—

(1) Immersion of young tillers in colchicine for 12 hr after cutting the top growth back to about 2 cm from the base.

(2) Immersion of similarly treated tillers in colchicine. Partial vacuum was applied for 3–4 min to remove air bubbles.

(3) Injection of colchicine into decapitated vigorously growing tillers with a hypodermic needle.

An excess amount of solution was used and the treatment was repeated daily for 4–6 days.

Of the plants treated by the above methods, 6 per cent. produced one seedhead per plant which exhibited normal anthesis (Figure 2). The pollen grains collected from these seedheads were regular in shape (Figure 3) and size and 69 per cent. were stainable. They set fertilized seed at the rate of 155 seeds per 100 cm of seedhead. The seeds have shown high germinating capacity on sterilized sand.

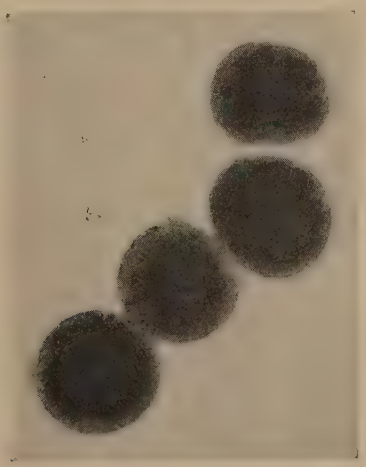

Fig. 3.—Well-formed stained pollen grains of Ronpha grass treated with colchicine.

There is no indication that any of the colchicine treatments used was superior in effect to the others. However, immersion in colchicine is a convenient method for the mass treatment of clonal material, especially when large numbers of plants are required for progeny evaluation.

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MITES ASSOCIATED WITH HONEYBEES IN QUEENSLAND

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In Queensland, casual infestations of honeybees, *Apis mellifera* L., by mites, *Pediculoides ventricosus* Newp.* and *Hypoaspis* sp.†, have been recorded (Veitch 1936, p. 93; Simmonds 1949, p. 42).

Recently during routine quarantine examinations mites determined as *Acarapis woodi* (Rennie) were found externally on a worker honeybee in a queen bee consignment from California, U.S.A. (Roff 1959). Following this, similar mites, stated to be *Acarapis dorsalis* or *Acarapis woodi externus* in the United States of America, have been located in various parts of North America, including California (Eckert 1959*a*, 1959*b*), Massachusetts (Morse 1960*a*), British Columbia and Alberta, and indirectly reported from Utah and Louisiana (Morse 1960*b*). Records also show that in 1926 *Acarapis externus* was recorded from the United States of America, Toronto (Eckert 1959*a*) and Quebec (Foote 1959).

Introductions into Queensland during 1960 from Italy, New Zealand, and United States of America (Louisiana, Mississippi and Tennessee) have been found to be infested on the thorax with *Acarapis* mites.

In Queensland, a quick survey has been made and *Acarapis* mites have been found on the thorax of worker honeybees from several Brisbane apiaries, and in several country areas extending from Warwick to Atherton. These widely spaced localities indicate that the mite must be of general distribution in the State. In no instance have *Acarapis* mites been found in or near the tracheae of honeybees in Queensland.

Acarapis woodi when found in the tracheae of honeybees is associated with the condition known as acarine (Isle of Wight) disease. There is an opinion that this mite is present but undetected in many countries, that it can exist as a harmless parasite, and that it only becomes pathogenic under particular circumstances (Morgenthaler 1959.) Recorded occurrences in countries other than Europe and North America are from Argentina, Chile, Uruguay, India, U.S.S.R. and Canary Islands (Jeffree 1959).

There is also an opinion that external mites on honeybees might be a variety of *A. woodi* (Baker *et al.* 1956, p. 62), or even a distinct species (Schneider 1941).

Assistance with identifications of mites found in Queensland has been given by the Commonwealth Institute of Entomology, London.

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* Now known as *Pyemotes ventricosus* (Newp.)

† Now determined as belonging to the genus *Neocypholaelaps.*

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